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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

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VOLUME XLV.

WOODS HOLE, MASS.

JULY TO DECEMBER, 1923

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BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY.

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I. TRUSTEES.

EX OFFICIO.

FRANK R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
*D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.
CORNELIA M. CLAPP, Mount Holyoke College, EMERITUS.

TO SERVE UNTIL 1926.

E. G. CONKLIN, Princeton University.
OTTO GLASER, Amherst College.
ROSS G. HARRISON, Yale University.
H. S. JENNINGS, Johns Hopkins University.
F. P. KNOWLTON, Syracuse University.
M. M. METCALF, Oberlin, Ohio.
WILLIAM PATTEN, Dartmouth College.
W. B. SCOTT, Princeton University.

*Deceased.

TO SERVE UNTIL 1925.

C. R. Crane, New York City, *President of the Corporation.*
I. F. Lewis, University of Virginia.
R. S. Lillie, Nela Research Laboratory.
E. P. Lyon, University of Minnesota.
C. E. McClung, University of Pennsylvania.
T. H. Morgan, Columbia University.
D. H. Tennent, Bryn Mawr College.
E. B. Wilson, Columbia University.

TO SERVE UNTIL 1924.

H. H. Donaldson, The Wistar Institute of Anatomy and Biology.
W. E. Garrey, Tulane University.
Caswell Grave, Washington University.
M. J. Greenman, The Wistar Institute of Anatomy and Biology.
*George Lefevre, University of Missouri, *Secretary of the Board.*
A. P. Mathews, The University of Cincinnati.
G. H. Parker, Harvard University.
C. R. Stockard, Cornell University Medical College.

TO SERVE UNTIL 1923.

H. C. Bumpus, Brown University.
R. A. Harper, Columbia University.
W. A. Locy, Northwestern University.
Jacques Loeb, The Rockefeller Institute for Medical Research.
George T. Moore, Missouri Botanical Garden, St. Louis.
L. L. Nunn, Telluride, Colorado.
W. J. V. Osterhout, Harvard University.
William M. Wheeler, Bussey Institution, Harvard University.

II. ACT OF INCORPORATION.

No. 3170

COMMONWEALTH OF MASSACHUSETTS.

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

---

*Deceased.

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand, Eight Hundred and Eighty-Eight.

[SEAL] HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY.

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee of finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT.[1]

Harvey S. Chase & Company,
Certified Public Accountants, 84 State Street, Boston.

January 22, 1923.

Mr. D. Blakely Hoar,
161 Devonshire Street,
Boston.

Dear Sir: We have completed our audit of the accounts of the Marine Biological Laboratory for the year ended December 31, 1922, as kept both at your office in Boston and at Woods Hole, and report thereon in the accompanying exhibits and schedules:

Exhibit A—Balance-Sheet as of December 31, 1922.

Exhibit B—Income-and-Expense for the Year ended December 31, 1922.

Schedule I—Investments (Book Values).

Schedule II—Cash Receipts and Disbursements on Account of Funds.

Schedule III—Land, Buildings, and Equipment.

Schedule IV—Supply Department Income-and-Expense Account for the Year ended December 31, 1922.

We certify that, subject to the comments herewith, the balance-sheet and income-and-expense statement shown in Exhibits A and B are in accordance with the books and correct, to the best of our knowledge and belief.

Very respectfully,

Harvey S. Chase & Company,
Certified Public Accountants.

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[1] Only a part of the audit is included in the Treasurer's Report. The complete audit is on file at the Laboratory and may be examined by any member.

Marine Biological Laboratory Balance Sheet, December 31, 1922.

Assets.

Cash:		
In bank	$ 1,592.05	
Petty cash fund	200.00	$ 1,792.05
Accounts receivable		14,535.47
Inventories:		
Supply department	23,960.63	
Biological Bulletin	3,329.25	27,289.88
Investments:		
Securities—Schedule I	12,151.12	
Less Loan	1,100.00	
	11,051.12	
Cash—Schedule II	516.64	11,567.76
Stock in General Biological Supply House, Inc.		12,700.00
Gansett property account	18,832.22	
Less mortgage	10,782.01	8,050.21
Educational Plant—Schedule III:		
Land	95,856.14	
Buildings	209,183.50	
Equipment	99,430.61	
	404,470.25	
Less Reserve for Depreciation	51,050.52	353,419.73
Items in suspense:		
First payment on purchase of Fish Estate, Woods Hole	5,000.00	
Expense for surveying and testing ground in re proposed extension of laboratory building	418.49	
Sundry items	32.16	5,450.65
Prepaid insurance		1,304.85
		$436,110.60

Liabilities.

Accounts payable		$ 2,611.66
Notes payable—Falmouth National Bank		10,000.00
Accrued charges (estimated)		1,200.00
Items in suspense		59.30
Trust funds		11,567.76
		$ 25,438.72
Balancing Account:		
Balance, January 1, 1922	$399,777.13	
Add:		
Balance of income account for year	5,894.75	
Special donation received from C. R. Crane for first payment on purchase of Fish Estate	5,000.00	410,671.88
		$436,110.60

Marine Biological Laboratory, Income & Expenses for Year Ended December 31, 1922.

	Expenses	Income	Loss	Gain
Administration expenses......	$ 9,570.85		$ 9,570.85	
Bar Neck Property expense...	318.00		318.00	
Biological Bulletin and annual dues.................	4,265.50	$ 3,653.31	612.19	
Biological Bulletin, adjustment of expenses for 1921...	200.21		200.21	
Carpenter department.......	958.18	1.55	956.63	
Chemical department........	2,012.79		2,012.79	
Dormitories................	2,384.01	2,660.78		$ 276.77
Instruction.................	6,866.46	9,390.00		2,523.54
Interest on notes payable.....	700.66		700.66	
Janitor's house expense......	7.84		7.84	
Library department.........	1,860.22		1,860.22	
Maintenance, buildings and grounds..................	6,640.42		6,640.42	
Mess......................	24,328.81	26,184.05		1,855.24
New laboratory.............	4,276.30		4,276.30	
Newman cottage............	80.13	150.00		69.87
Pumping station............	587.39		587.39	
Research department........	2,817.16	6,425.00		3,607.84
Sundry expense and income ..	804.11	10,853.61		10,049.50
Supply department (See Schedule IV)..................	44,004.65	49,368.07		5,363.42
Truck.....................	783.14		783.14	
Total current expenses...	$113,466.83		$28,526.64	
Total current income........	108,686.37	$108,686.37	23,746.18	$23,746.18
Excess of expenses.......	4,780.46		4,780.46	
Reserve for depreciation......	8,980.35			
Bad accounts written off....	365.97			
	$14,126.78			
Donations for expenses:				
Friendship Fund Inc.... $20,000.00				
Others..... 21.53	20,021.53			
Balance to balancing account............	$ 5,894.75			

Marine Biological Laboratory Investments, December 31, 1922.

Reserve Fund.

Cash on hand	$ 388.41	
* $3,000.00 American Telephone & Telegraph Company, 4's	2,921.25	
500.00 Western Telephone & Telegraph Company, 5's	496.88	
* 6 shares American Smelting & Refining Company, Preferred	732.00	
8 shares General Electric Company	907.25	
4 shares General Electric Company Special (par $10.00) received as a stock dividend.		
14 shares United Shoe Machinery Corporation, Preferred	393.75	
5 shares Massachusetts Gas Companies, Preferred	444.63	
	$6,284.17	
Items marked * are held as collateral on loan of	1,100.00	$5,184.17

Library Fund.

$300.00 U. S. Liberty Loan, First 4 1/4's	300.00	
4/5 of $1,000.00 American Telephone and Telegraph Company, 4's	779.00	
3 shares American Telephone & Telegraph Company	362.38	
3 shares General Electric Company	346.80	
1 1/2 shares General Electric Company Special (par $10.00) received as stock dividend.		
5 shares United Shoe Machinery Corporation, Preferred	140.63	
3 shares Massachusetts Gas Companies, Preferred	269.38	
*1 share American Smelting & Refining Company, Preferred	122.00	
	$2,320.19	
Less overdraft of cash	120.88	
		2,199.31

Lucretia Crocker Fund.

Cash on hand	249.11	
$300.00 U. S. Liberty Loan, 1st 4 1/4's	300.00	
1/5 of $1,000.00 American Telephone & Telegraph Company, 4's	194.75	
18 shares Vermont & Massachusetts Railroad Company	2,416.50	
3 shares General Electric Company	349.55	
1 1/2 shares General Electric Company Special (par $10.00) received as stock dividend.		
1 share Boston Elevated Second Preferred	133.00	
1 share American Telephone & Telegraph Company	120.79	
4 shares Boston Consolidated Gas Company, Preferred	420.58	4,184.28
Total, Exhibit A		$11,567.76

V. REPORT OF THE LIBRARIAN.

The growth of the Library continues steadily. The number of books added during the year was 473. Of these eighty volumes were received by purchase, 199 by binding of periodicals, 157 were gifts, and 21 were additions to the permanent loan from the American Museum of Natural History. Our valuable collection of reprints has been increased by the addition of 434 pamphlets.

The number of periodicals currently received was 229; of which 87 are received by subscription, 59 by exchange for the Biological Bulletin, 69 were gifts, and 14 were duplicates lent to us by the American Museum of Natural History. The total contents of the Library at the end of the year is 11,136 volumes and 9,393 pamphlets.

Two of the gifts received during the year are especially noteworthy. The first to be mentioned is the gift from Mrs. George Peirce of books selected from the working library of her husband, a distinguished physiologist and chemist, whose herioc death in 1919 at the height of a productive career of research has retarded the advance of biochemistry in America. This is a collection of thirty volumes, all recent books of importance, and is a very valuable addition to our library.

The second gift of special note is a complete set of the cards of the Concilium Bibliographicum. This is given by the Library of Carleton College through the good offices of Dr. Donnell B. Young of our Instructing Staff. These cards will be of very great use in the bibliographic research that is a prominent part of the work of investigators every summer. But before they can be used these cards must be filed in a suitable cabinet. Until a cabinet and room for it can be provided, the cards can only be stored.

Another notable gift is a collection of thirteen volumes of their publications given by Messrs. P. Blakiston's Son & Company through their representative, Mr. Horace G. White. These books, with one exception, are all published in 1919, or later, and all are desirable additions to the Library.

Dr. Christine Ladd-Franklin has presented to the Library the Concise Oxford Dictionary, edition of 1911, and a copy of Woodworth's Psychology, 1921. From Dr. R. P. Bigelow have been received several volumes, including the U. S. Entomological Commission Reports, Volumes 2 and 3, 1878–82.

Through the kindness of their authors we have received the following books:

B. M. Patton, "Laboratory Directions in Embryology," 5 volumes.
E. G. Conklin, "Direction of Human Evolution."
G. H. Parker, "Smell, Taste, and Allied Senses in Vertebrates."
W. C. Curtis, "Science and Human Affairs."
L. L. Woodruff, "Foundations of Biology."
B. H. Grave, "Birds of Wyoming."
W. A. Collier, "Einführung in die Variationsstatistik."
H. C. van der Heyde, "Physiology, Digestion, Respiration, and Excretion in the Echinoderms."
J. F. Nonides, "Herencia Mendeliana."
Libbie H. Hyman, "Laboratory Manual of Comparative Vertebrate Anatomy."
C. T. Brues, "Insects and Human Welfare."
A. H. Church, "Thallassophyta and Sub-aërial Transmigration."

When the present officers took over the administration of the Library, there was found a large accumulation of printed matter, mostly unbound, that was thought to be duplicates. During the early part of the year covered by this report, the Assistant Librarian spent considerable time sorting this material. Thirty-three volumes were found not to be duplicates and were added to the Library. They are included in the gifts mentioned in the first paragraph of this report. From the sale of 450 duplicates, we received $106.20, which was applied to the purchase of new books. There remain about 150 odd numbers and volumes and 1,000 reprints still to be catalogued.

The Director having authorized an extension of the exchange list, considerable time has been given to selecting and soliciting new exchanges with the Biological Bulletin. Nineteen new exchanges have been established, some of them bringing more than usually desirable publications, and in the following cases this includes back sets as well as current issues:

Hereditas,
Acta Zoologica,
Annalen des Naturhistorischen Museums, Vienna,
Archives Néerlandaises de Physiologie,
Carlsberg Laboratorium, Comptes Rendus,
Skandinavisches Archiv für Physiologie,
Svensk Botanisk Tidskrift, Vol. 1, 1907, to 16, 1922,
Kolloid Zeitschrift,
Revue générale des sciences pures et appliquées.

The completion of our sets of serial publications is one of the important aims of the library administration. It is, therefore, gratifying to report that we have been able to complete the following by purchase:

Allgemeine botanische Zeitschrift, Vols. 1–25,
Botanische Jahrbücher, Vols. 1–56,
Journal of Pharmacology and Experimental Therapeutics, Vols. 1–11,
Zeitschrift für Botanik, Vols. 1–13,
Zoological Record, Vols. 31–57.

The dictionary catalogue which was begun in 1920, now contains 11,051 cards, and work has started on the transfer of cards from the old classified catalogue.

The increase in the use of the Library has continued, the circulation during 1922 being about 1,200 items, besides 30 volumes borrowed from other libraries.

We still lack many reprints of work done at Woods Hole, and authors are requested to look over the reprints in the Library and to send us what are lacking. Among the special needs of the Library are a complete set of the *Philosophical Transactions of the Royal Society*, a good encyclopedia, and bibliographical aids, such as the Royal Society Catalogue of Scientific Papers, and several sections of the International Catalogue of Scientific Literature.

ROBERT P. BIGELOW,
Librarian.

VI. THE DIRECTOR'S REPORT.

To the Trustees of the Marine Biological Laboratory.

Gentlemen: I beg to submit herewith a report of the thirty-fifth session of the Marine Biological Laboratory for the year 1922.

The business of the Laboratory has become so complex, especially in the summer, that the executive officers can no longer assume the responsibility of administering it with reference only of major problems to the Board of Trustees once or twice during the year. The Executive Committee of the Board has accordingly been reorganized during the last two years to consist of the Director and Assistant Director *ex officiis* and three members of the Board resident in Woods Hole during the summer, one member being elected each year for a three-year period. The Committee meets weekly during the summer, and at other times of the year when called, and reports its principal activities at the annual meeting of the Board. In this way different members of the Board are successively brought into close contact with detailed problems of administration of the Laboratory. It is felt that this practice is good for the morale of the entire Institution; it is also a great relief to the administrative officers to share their responsibilities at times when decisions have to be made; naturally, also, it makes for greater steadiness and consistency in the conduct of affairs, through the establishment of precedents.

The season of 1922 was very gratifying as to attendance and all other evidences of active interest and wide coöperation in the affairs of the Laboratory.

Attendance.—The attendance of students in courses was 126, of investigators 182, making a total of 308 persons, who represented 104 institutions. These numbers are considerably in excess of any previous attendance, except in the case of students in courses whose numbers are limited by rule. Applications for working places were very considerably greater than could be granted; about 30 students had to be refused places on account of the rule limiting admission to courses, but it was possible to provide for nearly all applicants for research facilities. There is no doubt that if the present limitations in the Laboratory were removed, that numbers would mount much higher. For an institution devoted primarily to research, as ours is, this cannot be regarded as an end in itself. But there is no doubt that the present accommodations are inadequate to fulfill the present strictly justifiable needs of American Biology. This becomes more obvious year by year. If we are to continue to justify the status of the Marine Biological Laboratory as the national center of biological research we must accommodate ourselves to the idea of a future considerable increase in attendance. The investigator who desires quiet for his research must use the same rules of protection in the Marine Biological Laboratory that he does in the still larger university communities from which he generally comes.

The number of subscribing and coöperating institutions increased from 52 in 1921 to 61 listed on page 30; there were in addition three scholarship arrangements providing for five students. Of the total amount of $9,687.50 received from these institutions, $3,912.50 went for students' tables and $5,775.00 for research accommodations. In 1921 the receipts from subscribing and coöperating institutions amounted to $8,800.00. As 58 per cent. of the students paid their own fees in 1922 the fear that has been expressed lest subscribing and coöperating institutions should monopolize all the students' places does not appear to be justified. This is borne out in another way by comparing the total of institutions represented, 104, with the number of coöperating and subscribing institutions, 61. The growth in the number of these institutions is an encouraging sign of continued interest which represents the broad basis of support of the Laboratory.

The Report of the Treasurer (see p. 5).—The earned income of the Laboratory increased from $98,292.38 in 1921 to $108,686.37 in 1922; and the current expenses increased from $109,970.40 in 1921 to $113,466.83. The excess of current expenses over earned income was $11,678.02 in 1921 and only $4,780.46 in 1922. If it were not for the constant need for additions outside of current expenses the Laboratory would be approaching a self-supporting basis. The annual subvention of $20,000.00 a year from the Friendship Fund provides both for the excess of current expenses and also for necessary annual improvements and additions. The net assets of the Laboratory increased from $430,251.28 in 1921 to $436,110.60 in 1922; and the liabilities decreased from $30,474.15 in 1921 to $25,438.72 in 1922.

The increase of income over 1921 amounting to $10,393.99 is due to a relatively slight increase of the receipts of all departments and a large increase in the income of the Supply Department from $42,774.78 in 1921 to $49,368.07 in 1922.

Great encouragement in our plans for the new buildings proposed since 1919 was received during the year from the Rockefeller Foundation, from the Carnegie Corporation, and from Mr. C. R. Crane. Early in January the Board of the Rockefeller Foundation voted to authorize negotiations with the Marine Biological Laboratory on the basis of one-half of the total sum called for in our estimates, viz.: $1,000,000 for completing the Laboratory, library, and auditorium building and providing endowment for its maintenance. This was conditioned upon the total sum being raised, the continuance of the sum of $20,000 annually from the Friendship Fund, and making of satisfactory arrangements for reversion of endowment in case the work of the Laboratory were to be radically changed or abandoned. In February the Trustees of the Carnegie Corporation appropriated the sum of one hundred thousand dollars ($100,000) to the Marine Biological Laboratory for the purpose of creating a fund the income of which shall be used for the maintenance of its buildings and laboratories provided gifts to the total amount of one million dollars ($1,000,000) be secured for the joint purpose of providing for buildings and for their maintenance. Mr. Crane also assured the Director that the maintenance of $20,000 a year from the Friendship Fund would be guaranteed as soon as the other conditions were met. Circumstances during the remainder of the year did not permit an active campaign to secure the additional funds needed though inquiries have been made that should inform us during the session of 1923 just what efforts will be required.

During the summer farther study of the building plans and of equipment was made and incorporated in working drawings and specifications by the architects. Costs of building having increased since the original estimates were prepared in March 1922, the necessity of some curtailment of building plans was considered, and alternative schemes for constructing major parts of the entire plan were also prepared by the architects. The plans and specifications are ready for the builders as soon as the balance of the sum needed to meet the conditions has been provided.

The Laboratory traces its origin directly back to the impetus given the study of Marine Biology by the establishment of the Anderson School of Natural History on Penikese Island near Woods Hole in 1873 by Louis Agassiz. As the summer of 1923 will be the fiftieth anniversary of this epoch-making event in the history of biology in America, it seemed to be an appropriate time for the establishment of a memorial in memory of this service of Agassiz. On recommendation of Dr. Clapp a committee was appointed by the Trustees to consider and recommend plans for the establishment of an Agassiz Memorial. It is hoped that this may take the form of a suitable monument on the site of Agassiz's laboratory.

Towards the end of the year a most important addition to the real estate holdings of the Laboratory was made by the President of the Board, Mr. C. R. Crane, who presented to the Laboratory the property known as the Kidder House at the corner of East and Water Streets immediately adjoining the old buildings of the Laboratory and forming part of the block on which the first buildings of the Laboratory were erected. We owe a debt of gratitude to Mrs. Kidder as well as to Mr. Crane for the acquisition of this land, which she may be said to have held for the Laboratory pending a favorable opportunity for its acquisition. In the Resolution concerning the death of Mr. Camillus G. Kidder, included in this report, reference is made to the interest of the Kidder family in plans for biological work at Woods Hole at a period even ante-dating the establishment of the Marine Biological Laboratory. It is to that interest that we owe our most important sites, including the land on which the Crane laboratory stands; the present status of the Laboratory is in an important respect the fruition of the far-sightedness of the Kidder family.

The Trustees at their meeting on August 8, 1922, established a class of emeritus trustees to honor Dr. Cornelia M. Clapp who expressed her desire to be relieved from membership on the Board after twenty-two years of service. This action was taken in acknowledgment of the exceptional spirit of devotion displayed toward the laboratory from its foundation to the present time by Miss Clapp and in the confident expectation of her continued interest. Dr. Clapp was accordingly elected Trustee Emeritus at the meeting of the Board in August 1922.

At the meeting of the Corporation August 8, 1922, two new members were elected to the Board of Trustees to fill vacancies in the newly elected class of 1926: Professor Otto Glaser of Amherst College, and Professor F. P. Knowlton of Syracuse University, both old members of the Corporation and regular investigators at the Marine Biological Laboratory.

At the meeting both of the Trustees and also of the Corporation the deaths of three former Trustees of the Laboratory were commemorated in the following memorials:

Resolution on the death of William T. Sedgwick, drawn up by Dr. Cornelia M. Clapp:

William T. Sedgwick, professor of biology and public health at the Massachusetts Institute of Technology, Boston, died on January 25, 1921.

The career of Professor Sedgwick as a teacher is well known. He was a fluent lecturer, and as one of his students has said he could make science popular and could take subjects of popular interest and clothe them in the language of science. Soon after he came to Boston he became consulting biologist of the State Board of Health and after that was identified with the interests of the American Public Health Association. He has been called the "Ambassador of Health." It is, however, as a member of the Board of Trustees of the Marine Biological Laboratory that we remember him today. In 1887 came an awakening of interest in a project for starting a sea-side laboratory, and his aid was sought by the Woman's Education Association in their efforts in this direction.

It was proposed that there should be a permanent institution, incorporated, and supported by the educational institutions of the country. There is no name more prominent in this movement than that of Professor Sedgwick. He was one of the original seven trustees, served on committees, suggested and helped carry out plans for raising funds, enlisted the aid of benevolent Bostonians, and not least among his activities was the effort to master the practical details concerned with the living conditions in Woods Hole.

He spent the summer of 1888 in this vicinity looking after the interests of the Laboratory. His devotion to the Laboratory during the earliest and most critical period of its history will be remembered and cherished by the friends of the Laboratory. A life-long friend, Dr. E. B. Wilson, has written an appreciation which it is hoped will find a place among the records of the Marine Biological Laboratory.

Resolution on the death of Camillus G. Kidder, drawn up by Dr. E. B. Wilson:

The Trustees of the Marine Biological Laboratory record with deep regret the death of Camillus G. Kidder, a member of this Board since 1897, and always one of its highly valued friends and loyal supporters. Both Mr. Kidder and his brother, Dr. Jerome H. Kidder, were among the earliest of the summer residents at Woods Hole, and both were from the beginning in close touch with the biological work here carried on, through their close friendship with Spencer F. Baird and his associates in the U. S. Fish Commission. From the first Mr. Kidder showed a sympathetic interest in Baird's plans for the larger development of that work. A conspicuous example of this, and one that should have a place in the annals of the Marine Biological Laboratory, was the early purchase by himself and Dr. Kidder, at Baird's suggestion, of the land on which the new laboratory now stands, in order to hold it in friendly hands with a view to the possible later development of Baird's plans. When the Marine Biological Laboratory was established at Woods Hole in 1888, Mr. Kidder unhesitatingly extended his interest to the new enterprise, becoming a steadfast supporter of its plans for scientific work and for friendly coöperation with the Fish Commission. Years later it was through him and Mrs. Jerome Kidder that Baird's foresight brought fruit through the acquisition by the Marine Biological Laboratory of the Kidder Land, later to become the site of the Crane Laboratory, and the future site, as we hope, of its further extension.

The Trustees here record their appreciation of Mr. Kidder's wise counsel in the conduct of the Laboratory and of the kindly and understanding spirit in which he took part in the deliberations of this Board and from time to time presided over its meetings. We cherish the memory of his sympathetic personality and generous friendship, and we are grateful for the long continued services that he rendered.

Resolution on the death of Alfred G. Mayor, drawn up by Dr. E. G. Conklin:

The death of Dr. A. G. Mayor, Director of the Department of Marine Biology of the Carnegie Institution of Washington at his laboratory at Tortugas, Florida, on June 24 last is lamented as a serious loss by the Marine Biological Laboratory. Dr. Mayor was not only a distinguished organizer and director of research in tropical marine biology and the leading American student of Cœlenterata but he was ever the loyal friend of the Marine Biological Laboratory. He carefully planned the work of his Department of the Carnegie Institution so as to supplement and not to duplicate or interfere with the work of this Laboratory, and at a critical period in our history he said that he would gladly abandon his cherished projects if by so doing he could materially aid the Marine Biological Laboratory. He was a useful member of our Board of Trustees and he assisted in the work of our institution not only by his attendance and lectures but also by furnishing facilities and generous assistance for investigations in tropical waters to members of our Staff and Corporation.

It is especially as a helpful, unselfish, and genial friend that we love to remember him; he was always ready to sacrifice himself for the good of others and it can be truly said that he gave his life not merely to science but also to his fellow scientists.

The Marine Biological Laboratory expresses to his family its deep sympathy in their bereavement and its high appreciation of his work and character.

There is attached as part of this report a list of the Staff and of Investigators and Students for 1922, a tabular view of attendance 1918–1922, lists of subscribing institutions, of the Evening Lectures and of the Members of the Corporation.

I. THE STAFF.

1922.

Frank R. Lillie, *Director*, Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

Gilman A. Drew, *Assistant Director*, Marine Biological Laboratory.

ZOÖLOGY.

I. INVESTIGATION.

Gary N. Calkins, Professor of Protozoölogy, Columbia University.

E. G. Conklin, Professor of Zoölogy, Princeton University.

Caswell Grave, Professor of Zoölogy, Washington University.

H. S. Jennings, Professor of Zoölogy, Johns Hopkins University.

George Lefevre, Professor of Zoölogy, The University of Missouri.

Frank R. Lillie, Professor of Embryology, The University of Chicago.

C. E. McClung, Professor of Zoölogy, University of Pennsylvania.

S. O. Mast, Professor of Zoölogy, Johns Hopkins University.

T. H. Morgan, Professor of Experimental Zoölogy, Columbia University.

G. H. Parker, Professor of Zoölogy, Harvard University.

E. B. Wilson, Professor of Zoölogy, Columbia University.

II. INSTRUCTION.

Robert H. Bowen, Instructor in Zoölogy, Columbia University.

Edward F. Adolph, Instructor in General Physiology, University of Pittsburgh.

J. A. Dawson, Assistant Professor of Biology, Dalhousie University.

Ann H. Morgan, Professor of Zoölogy, Mount Holyoke College.

C. L. PARMENTER, Instructor in Zoölogy, University of Pennsylvania.

J. PAUL VISSCHER, Instructor in Zoölogy, Washington University.

BENJAMIN P. YOUNG, Assistant Professor of Zoölogy, Cornell University.

DONNELL B. YOUNG, Assistant Professor of Biology, Carleton College.

PROTOZOÖLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.

LOUISE H. GREGORY, Assistant Professor of Zoölogy, Columbia University. (Absent on leave.)

FLORENCE DEL. LOWTHER, Instructor in Zoölogy, Barnard College.

EMBRYOLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

HUBERT B. GOODRICH, Associate Professor of Zoölogy, Wesleyan University.

BENJAMIN H. GRAVE, Professor of Biology, Wabash College.

ROBERT S. MCEWEN, Assistant Professor of Zoölogy, Oberlin College.

HAROLD H. PLOUGH, Associate Professor of Biology, Amherst College.

CHARLES G. ROGERS, Professor of Comparative Physiology, Oberlin College. (Absent on leave.)

ELIZABETH A. SMITH, Assistant Professor of Zoölogy, University of Wisconsin.

PHYSIOLOGY.

I. INVESTIGATION.

HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.

WALTER E. GARREY, Professor of Physiology, Tulane University.

RALPH S. LILLIE, Biologist, Nela Research Laboratory, Department of Pure Science, Nela Park, Cleveland, Ohio.

ALBERT P. MATHEWS, Professor of Biochemistry, The University of Cincinnati.

II. INSTRUCTION.

MERKEL H. JACOBS, Assistant Professor of Zoölogy, University of Pennsylvania.

FRANK P. KNOWLTON, Professor of Physiology, Syracuse University.

Alfred C. Redfield, Assistant Professor of Physiology, Harvard Medical School.

Reynold A. Spaeth, Associate in Physiology, School of Hygiene and Public Health, Johns Hopkins University.

BOTANY.

I. INVESTIGATION.

S. C. Brooks, Department of Public Health, Washington, D. C.

Edward M. East, Professor of Experimental Plant Morphology, Harvard University.

Robert A. Harper, Professor of Botany, Columbia University.

E. Newton Harvey, Assistant Professor of Physiology, Princeton University.

Winthrop J. V. Osterhout, Professor of Botany, Harvard University.

II. INSTRUCTION.

Ivey F. Lewis, Professor of Biology, University of Virginia.

William Randolph Taylor, Instructor in Botany, University of Pennsylvania.

William H. Weston, Jr., Assistant Professor of Cryptogamic Botany, Harvard University.

LIBRARY.

Robert P. Bigelow, Librarian and Associate Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology. Librarian.

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Assistant Librarian.

CHEMICAL SUPPLIES.*

Oliver S. Strong, Associate Professor of Neurology, Columbia University, New York City, Chemist.

SUPPLY DEPARTMENT.

George M. Gray, Curator.

Thomas M. Douthart, Assistant Curator.

John J. Veeder, Captain.

E. M. Lewis, Engineer.

A. W. Leathers, Head of Shipping Department.

A. M. Hilton, Collector.

J. McInnis, Collector.

F. M. MacNaught, Business Manager.

2. INVESTIGATORS AND STUDENTS, 1922.

INDEPENDENT INVESTIGATORS—Zoölogy.

ADAMS, A. ELIZABETH, Associate Professor of Zoölogy, Mount Holyoke College.

ADDISON, WILLIAM H. F., Professor of Histology and Embryology, University of Pennsylvania.

ADOLPH, EDWARD F., Instructor in Physiology, University of Pittsburgh.

ANDERSON, ERNEST G., Research Associate, Carnegie Institution, Cold Spring Harbor.

BASCOM, KELLOGG F., University of Chicago.

BIGELOW, ROBERT P., Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology.

BISHOP, MABEL, Professor of Zoölogy, Hood College.

BODINE, JOSEPH H., Instructor in Zoölogy, University of Pennsylvania.

BOWEN, ROBERT H., Instructor in Zoölogy, Columbia University.

BRIDGES, CALVIN B., Research Assistant, Carnegie Institution of Washington.

BUDINGTON, ROBERT A., Professor of Zoölogy, Oberlin College.

CALKINS, GARY N., Professor of Protozoölogy, Columbia University.

CAROTHERS, E. ELEANOR, University of Pennsylvania.

CHAMBERS, ROBERT, Professor of Histology and Embryology, Cornell University Medical College.

CHARLTON, HARRY H., Assistant Professor of Anatomy, University of Missouri.

CLAPP, CORNELIA M., Professor Emeritus of Zoölogy, Mount Holyoke, College.

CLARK, ELIOT R., Professor of Anatomy, University of Missouri.

CLARK, ELEANOR L., Columbia, Mo.

COLE, WILLIAM H., Professor of Biology, Lake Forest College.

CONKLIN, EDWIN G., Professor of Biology, Princeton University.

COPELAND, MANTON, Professor of Biology, Bowdoin College.

COWDRY, EDMUND V., Associate Member, Rockefeller Institute.

COWDRY, NATHANIEL H., 1142 Madison Ave., New York City.

DANCHAKOFF, VERA, Assistant Professor of Anatomy, College of Physicians and Surgeons.

DAWSON, JAMES A., Professor of Biology, Dalhousie University.

DOLLEY, DAVID H., Professor, University of Missouri.

DOLLEY, WILLIAM L., JR., Professor of Biology, Randolph-Macon College.

DONALDSON, HENRY H., Professor of Neurology, Wistar Institute.

DREW, GILMAN A., Assistant Director, Marine Biological Laboratory, Woods Hole, Mass.

GLASER, OTTO, Professor of Biology, Amherst College.

GOLDFARB, A. J., Assistant Professor, College of the City of New York.

GOLDSMITH, WILLIAM M., Professor of Biology, Southwestern College.

GOODRICH, H. B., Associate Professor of Zoölogy, Wesleyan University.

GRAVE, BENJAMIN H., Professor of Zoölogy, Wabash College.

HEILBRUNN, LEWIS V., Assistant Professor of Zoölogy, University of Michigan.

HESS, WALTER N., Professor of Biology, DePauw University.

HIBBARD, HOPE, Associate Professor of Zoölogy, Elmira College.

HOWE, HARRISON E., 2702 36th St., N. W., Washington, D. C.

HUETTNER, ALFRED F., Instructor, Columbia University.
HUMPHREY, RUFUS R., Instructor, Cornell University.
JACOBS, MERKEL H., Assistant Professor of Zoölogy, University of Pennsylvania.
JENNINGS, HERBERT S., Professor of Zoölogy, Johns Hopkins University.
JUST, ERNEST E., Professor of Zoölogy, Howard University.
KNOWER, HENRY McE., Professor of Anatomy, University of Cincinnati.
LANCEFIELD, DONALD E., Assistant Professor of Zoölogy, Columbia University.
LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri.
LEWIS, WARREN H., Collaborator, Carnegie Institution of Washington.
LEWIS, MARGARET R., Collaborator, Carnegie Institution of Washington.
LILLIE, FRANK R., Chairman, Department of Zoölogy, University of Chicago.
LYNCH, RUTH S., Assistant, Johns Hopkins University.
McCLUNG, CLARENCE E., Director of Zoölogical Laboratory, University of Pennsylvania.
McEWEN, ROBERT S., Assistant Professor of Zoölogy, Oberlin College.
MADDOCK, STEPHEN J., Teaching Fellow in Histology, Harvard University Medical School.
MALONE, EDWARD F., Professor of Histology, University of Cincinnati.
MARTIN, EARL A., Assistant Professor, College of the City of New York.
MAST, SAMUEL O., Professor of Zoology, Johns Hopkins University.
METZ, CHARLES W., Member of Staff, Carnegie Institution, Cold Spring Harbor.
MOORE, EMILY L., Research Fellow, Yale University.
MORGAN, THOMAS H., Professor of Experimental Zoölogy, Columbia University.
MORGAN, ANN H., Professor of Zoölogy, Mount Holyoke College.
MORGAN, LILIAN V., 409 West 117th St., New York City.
NOYES, BESSIE, North Carolina College for Women.
PARKER, GEORGE H., Professor of Zoölogy, Harvard University.
PARMENTER, CHARLES L., Instructor, University of Pennyslvania.
PATTEN, WILLIAM, Professor of Biology, Dartmouth College.
PLOUGH, HAROLD H., Associate Professor of Biology, Amherst College.
POTTS, FRANK A., Lecturer in Zoölogy, University of Cambridge.
SCHRADER, FRANZ, Bryn Mawr College.
SHUMWAY, WALDO, Assistant Professor of Biology, Dartmouth College.
SIVICKIS, P. B., University of Chicago.
SMITH, ELIZABETH A., Assistant Professor of Zoölogy, University of Wisconsin.
SPAULDING, EDWARD G., Professor of Philosophy, Princeton University.
SPEIDEL, CARL C., Assistant Professor of Anatomy, University of Virginia.
STARK, MARY B., Professor of Embryology and Histology, New York Hospital Medical College.
STRONG, OLIVER S., Associate Professor of Neurology, Columbia University.
STURTEVANT, ALFRED H., Research Assistant, Carnegie Institution of Washington.
SWETT, FRANCIS H., Instructor in Anatomy, Johns Hopkins University Medical School.
SWINGLE, WILBUR W., Assistant Professor of Biology, Yale University.
SYKES, GEORGE F., Teaching Fellow, Harvard University Medical School.
TENNENT, DAVID H., Professor of Biology, Bryn Mawr College.
TRACY, HENRY C., Professor of Anatomy, University of Kansas.
WEINSTEIN, ALEXANDER, Research Fellow, Columbia University.
WIEMAN, HARRY L., Professor of Zoölogy, University of Cincinnati.
WOODRUFF, LORANDE L., Professor of Biology, Yale University.

Woodward, Alvalyn E., Amherst College.
Young, Benjamin P., Assistant Professor of Zoölogy, Cornell University.
Young, Donnell B., Assistant Professor of Biology, Carleton College.

BEGINNING INVESTIGATORS—Zoölogy.

Bean, Raymond J., Instructor in Biology, Western Reserve University.
Bennitt, Rudolph, Teaching Fellow, Harvard University.
Brailey, Miriam E., Assistant in Embryology, Mount Holyoke College.
Brown, Alice L., Assistant in Anatomy, Cornell University Medical College.
Chace, Eunice E., Instructor, Smith College.
Chase, Ruth W., Assistant in Zoölogy, University of Wisconsin.
Cole, Elbert C., Trinity College.
Emerson, Sterling H., Carnegie Institution, Cold Spring Harbor.
Ferry, Ruth M., Assistant, Carnegie Institution, Cold Spring Harbor.
Fry, Henry J., Columbia University.
Gilson, Arthur S., Jr., Research Student, Harvard University.
Hayden, Margaret A., Instructor in Zoölogy, Wellesley College.
Hinrichs, Marie A., University of Chicago.
Hoadley, Leigh, Assistant, University of Chicago.
Johnson, H. Herbert, Laboratory Assistant in Zoölogy, Columbia University.
Johnson, George E., Graduate Student, Harvard University.
Lackey, James B., Instructor in Zoölogy, Mississippi College.
Lowman, Edith, Assistant Instructor, Yale University.
Lowther, Florence deL., Instructor, Barnard College.
MacBride, Lavinia G., Graduate Student, University of Michigan.
MacDougall, Mary S., Head of Biology Dept., Agnes Scott College.
Moses, Mildred S., Assistant, Carnegie Institution, Cold Spring Harbor.
Overstreet, Mrs. Harry A., 802 West 181st St., New York City.
Smith, Christianna, Graduate Fellow, Cornell University.
Sturtevant, Phoebe R., Carnegie Institution of Washington.
Tharaldsen, Conrad E., Assistant Professor of Zoölogy, Northwestern University,
Thatcher, Lloyd E., Instructor in Zoölogy, University of Michigan.
Uhlemeyer, Bertha, Instructor in Zoölogy, Washington University.
Varian, Basil B., Columbia University.
Vicari, Emilia, Columbia University.
Visscher, J. Paul, Fellow, Washington University.
Wallace, Edith M., Carnegie Institution.
Warren, Herbert S., Assistant in Zoölogy, Columbia University.
Williams, Stephen C., Wesleyan University.

INDEPENDENT INVESTIGATORS—Physiology.

Bishop, George H., Associate, Washington University Medical College.
Bradley, Harold C., Professor of Physiological Chemistry, University of Wisconsin.
Clowes, George H. A., Research Director, Eli Lilly & Co., Indianapolis, Ind.
Collett, Mary E., Instructor in Physiology, University of Buffalo.
Cohn, Edwin J., Assistant Professor of Physiological Chemistry, Harvard University Medical School.
Denis, Willy, Associate Professor of Physiological Chemistry, Tulane University.

Edwards, Dayton J., Associate Professor of Physiology, Cornell University Medical College.
Garrey, Walter E., Professor of Physiology, Tulane University.
Geiser, Samuel W., Assistant Professor of Zoölogy, Washington University.
Greisheimer, Esther M., Instructor in Physiology, University of Minnesota.
Harvey, E. Newton, Professor of Physiology, Princeton University.
Hecht, Selig, National Research Council Fellow, Harvard University.
Hitchcock, David I., Assistant, Rockefeller Institute for Medical Research.
Hopkins, Hoyt S., Assistant Professor of Physiology, Baylor University Medical College.
Irwin, Marian, Research Worker, Radcliffe College.
Knowlton, Frank P., Professor of Physiology, Syracuse University.
Lillie, Ralph S., Biologist, Nela Research Laboratories, Cleveland, Ohio.
Loeb, Jacques, Head of Division of Experimental Biology, Rockefeller Institute for Medical Research.
Loeb, Leo, Professor of Comparative Pathology, Washington University.
Moore, Arthur R., Professor of Physiology, Rutgers College.
Morgulis, Sergius, Professor of Biochemistry, University of Nebraska, College of Medicine.
Pond, Samuel E., Biologist, Nela Research Laboratories, Cleveland, Ohio.
Rapport, Anne Y., Associate in Physiology, Bryn Mawr College.
Redfield, Alfred C., Assistant Professor of Physiology, Harvard University Medical School.
Smith, Homer W., Eli Lilly & Co., Indianapolis, Ind.
Spaeth, Reynold A., Associate in Physiology, School of Public Health, Johns Hopkins University.
Sparrow, Carroll M., Professor of Physics, University of Virginia.
Warren, Howard C., Professor of Psychology, Princeton University.

BEGINNING INVESTIGATORS—Physiology.

Bierman, Jessie M., Medical Student, University of Chicago.
Bright, Elizabeth M., Research Assistant in Physiology, Harvard University Medical School.
Burlingham, Robert, 156 East 66th St., New York City.
Cattell, Ware, Garrison, N. Y.
Hendry, Jessie L., Technician, Harvard University Medical School.
Klopp, John W., University of Pennsylvania, School of Medicine.
Page, Irvine H., Chemist, Eli Lilly & Co., Indianapolis, Ind.
Sampson, Myra M., Assistant Professor of Zoölogy, Smith College.
Shepard, Charles E., Teaching Fellow, University of Minnesota.
Studebaker, Mabel T., Eli Lilly & Co., Indianapolis, Ind.
Walden, Eda B., Chemist, Eli Lilly & Co., Indianpaolis, Ind.

INDEPENDENT INVESTIGATORS—Botany.

Brooks, Sumner C., Hygienic Laboratory, United States Public Health Service.
Brooks, Mrs. S. C., Hygienic Laboratory, United States Public Health Service.
Cleland, Ralph E., Assistant Professor of Biology, Goucher College.
East, Edward M., Professor of Experimental Plant Morphology, Harvard University.

Kylin, Harold, Lund, Sweden.
Lewis, Ivey F., Professor of Biology, University of Virginia.
Lyman, George R., U. S. Department of Agriculture, Washington, D. C.
Moore, George T., Director Missouri Botanical Gargen, St. Louis, Mo.
Osterhout, Winthrop J. V., Professor of Botany, Harvard University.
Phillips, Everett F., U. S. Department of Agriculture, Washington, D. C.
Ray, George B., Instructor in Applied Physiology, Harvard University Medical School.
Schramm, J. R., Professor of Botany, Cornell University.
Snow, Laetitia M., Associate Professor of Botany, Wellesley College.
Taylor, William R., Assistant Professor of Botany, University of Pennsylvania.
Weston, William H., Jr., Assistant Professor of Botany, Harvard University.

BEGINNING INVESTIGATORS—Botany.

Cook, Sherburne F., Teaching Fellow, Harvard University.
Harris, Earl S., Harvard University.
Harvey, Paul A., Teaching Fellow, Harvard University.
Keefe, Anselm M., Instructor, St. Norbert's College.
Lyon, Charles J., Instructor in Biology, Dartmouth College
MacInnes, Jean, Massachusetts Institute of Technology.
MacKaye, Robert K., Student, Harvard University.

STUDENTS.

1922.

BOTANY.

Bristol, Mary L., Student, Connecticut College.
Easton, Charlotte, Head of Biology Dept., Skidmore College.
Hess, Florence G., Student, Cornell University
McNair, Mrs. George T., Chickasha, Okla.
Nugent, Gertrude V., Teacher, East Boston, Mass.
Page, Helen D., Student, University of Chicago.
Smith, Fanny Fern, Student, Washington University
Woodhead, Arthur E., Professor of Biology, Western Maryland College.

EMBRYOLOGY.

Anderson, Pearl, Assistant in Zoölogy, Vassar College.
Bradley, Lillian H., Columbia University.
Brown, Louise K., Student, Agnes Scott College.
Butler, Elmer G., Instructor in Zoölogy, University of Vermont.
Chamberlain, William H., Columbia University.
Chang, C., Student, Harvard Medical School.
Hallauer, Emily E., Swarthmore College.
Halter, Clarence R., Instructor in Zoölogy, New York University.
Hawkins, Mary O'Neil, 1331 Columbine St., Denver, Colorado.
Hulpieu, Harold R., Student, Southwestern College.
Levy, Joseph, Student, Johns Hopkins University.
Lillie, Margaret H., Student, Mount Holyoke College.

Lucas, Alfred M., Student, Wabash College.
McCaa, Fanny, Instructor, Agnes Scott College.
Metcalf, Rachel V., Instructor in Zoölogy, Mount Holyoke College.
Miller, Harry M., Jr., Fellow in Zoölogy, University of Illinois.
Miller, Franklin R., Student, Illinois Wesleyan University
Pierson, Charles J., Professor of Zoölogy, University of Maryland.
Robinson, Owen L., Student, De Pauw University.
Shaver, Jesse M., Assistant Professor of Biology, George Peabody College.
Steen, Edwin B., Assistant in Zoölogy, Wabash College.
Stevens, Edith, West Virginia University.
Stewart, Colin C., Jr., Student, Dartmouth College.
Stewart, Dorothy R., Assistant, Washington University.
Towler, Viola, Shorter College.
Wassum, Eva E., Student, Agnes Scott College.
Wicks, Nina A., Student, Knox College.
Wilcox, Gladys, University of Delaware.

PHYSIOLOGY.

Brown, Madelaine R., Social Worker, State Hospital for Mental Diseases, Howard, R. I.
Brown, Mary J., Associate Professor, Transylvania College.
Coventry, Frances A., Assistant, Biology Dept., Goucher College.
Dickinson, Porter S., Student, Harvard Medical School.
Dimick, G. Priscilla, Smith College.
Drake, Dorothy, Assistant in Physiology, Mount Holyoke College.
Flexner, Louis B., Student, University of Chicago.
Gilman, Charlotte W., Instructor in Zoölogy, Vassar College.
Hartman, Arthur M., 1414 Girard St., N. W., Washington, D. C.
Iszard, Miriam S., Instructor in Bacteriology, University of Pennsylvania.
Landis, Eugene M., Student, University of Pennsylvania.
Lewton, Lucy O., Barnard College.
Lindsay, Blanche, Assistant in Zoölögy and Physiology, Wellesley College.
Morrison, Thomas F., Student, Princeton University.
Patch, Esther M., Teacher, Stoneham, Mass.
Reynolds, Philip A., Boston University School of Medicine.
Sloan, Louise L., Student, Bryn Mawr College.
White, E. Grace, Professor of Biology, Shorter College.
Woollard, Herbert H., Lecturer in Anatomy, University College, London.

PROTOZOÖLOGY.

Baker, W. B., Assistant Professor of Biology, Emory University.
Box, Cora M., Assistant Professor, University of Cincinnati.
Boyd, George H., Instructor in Biology, Emory University.
Coutant, Mary W., Instructor in Botany, Barnard College.
Emmart, Emily W., 817 N. Fremont Ave., Baltimore, Md.
Graves, Isabelle A., Assistant Bacteriologist, City Laboratory, New Haven, Conn.
Noland, Lowell E., Instructor, Zoology Dept., University of Wisconsin.
Schrader, Sally H., Bryn Mawr, Pa.

Tao, Wei Sun, Student, Columbia University.
Tilden, Evelyn B., Rockefeller Institute.
Unger, W. Byers, Instructor in Biology, Lafayette College.
Walters, Mary J., Instructor, Goucher College.

ZOÖLOGY.

Anderson, Ethel L., University of Kentucky.
Anderson, Hope E., Student, Mount Holyoke, College.
Bamber, Maurine, Student, Knox College.
Baton, Gertrude M., Assistant Instructor in Biology, Carnegie Institute of Technology.
Brown, Bernice D., Student, Oberlin College.
Buehler, Eugene O., Student, Wabash College.
Burwell, Margaret S., Student, Sweet Briar College.
Campbell, Eva G., Instructor, North Carolina College for Women.
Cooper, Drury W., Jr., Student, Rutgers College.
Copenhaver, Wilfred M., Assistant in Biology, Yale University.
Denby, Edwin O., Student, Harvard College.
Dixon, Perrine C., Student, Sophie Newcomb College.
Dooley, Parker, Student, Illinois Wesleyan University.
Faw, Helen A., Student, Agnes Scott College.
Federighi, Henry, Student, Rutgers College.
Fuller, Andrew B., Student, University of Pennsylvania.
Gorham, Grace V., Undergraduate student, Mount Holyoke College.
Grant, Jean F., Student, Sweet Briar College.
Gray, Irving E., Instructor, DePauw University.
Gray, Nina E., Student, DePauw University.
Hagedon, Edith L., Student, DePauw University.
Hall, Frank G., Assistant in Zoölogy, University of Wisconsin.
Hopkins, Aubrey E., College of William and Mary.
Houghton, Dorothy, Student, Barnard College.
Huber, Ernst, Associate in Anatomy, Johns Hopkins Medical School.
Hunsicker, Mary G., Student, University of Pennsylvania.
Jones, Myrna F., Student, Doane College.
Kennan, Ada B., Assistant in Zoölogy, University of Michigan.
Kubicek, Helen M., Student, Doane College.
Lewis, Marion F., Student, Mount Holyoke College.
Luce, Robert H., Carleton College.
Maguire, Gertrude A., Student, Hunter College.
Mason, Karl E., Assistant in Zoölogy, Yale University.
McNair, George T., Professor of Zoölogy, Oklahoma College for Women.
Miehling, Ruth B., Student, Hunter College.
Miller, Glenn O., Student, Southwestern College.
Morgan, Ruby M., Student, Oberlin College.
Nickell, Faith E., Washington University.
Norton, Hermon, Student, Wesleyan University.
Nyi, Tsung-tsong, Student, Smith College.
Payne, Mary G., Student, Butler College.
Perry, Lydia S., Student, Oberlin College.
Pritchard, Maybelle, Student, Radcliffe College.

Reed, Lucile L., Student, Sophie Newcomb College.
Riley, Philip L., Student, Massachusetts Institute of Technology.
Rose, Bertha E., Assistant in Zoölogy, University of Wisconsin.
Rustia, Constancio P., Graduate Student, University of Chicago.
Sampson, Howard J., Massachusetts Agricultural College.
Scott, Gordon H., Assistant, Johns Hopkins University.
Soisson, Mary C., Goucher College.
Stevens, Dorothy H., Student, Connecticut College.
Stiffler, Ethel G., Student, Goucher College.
Strauss, Maurice B., Student, Amherst College.
Sumwalt, Margaret, Student, Goucher College.
Taft, Charles H., Jr., Instructor in Biology, Tufts College.
Taylor. Elizabeth R., University of Delaware.
Theocharides, Electra, Assistant in Biology, Constantinople College.
Tingley, Mary A., Instructor, Shorter College.
Van Horn, Amey D., Instructor in Biology, Alfred University.

3. TABULAR VIEW OF ATTENDANCE.

	1918	1919	1920	1921	1922
INVESTIGATORS—Total	93	134	136	172	182
Independent:					
Zoölogy	51	68	69	79	87
Physiology	16	24	22	26	28
Botany	5	7	7	13	15
Under Instruction:					
Zoölogy	16	21	29	34	34
Physiology	3	10	7	11	11
Botany	2	4	2	9	7
STUDENTS—Total	69	128	120	120	126
Zoölogy	41	55	56	53	59
Protozoölogy	—	15	15	11	12
Embryology	12	33	26	28	28
Physiology	10	17	15	18	19
Botany	6	8	8	10	8
TOTAL ATTENDANCE	162	262	256	292	308
INSTITUTIONS REPRESENTED—					
Total	72	88	86	95	104
By investigators	49	61	55	67	71
By students	38	62	57	53	68
SCHOOLS AND ACADEMIES REPRESENTED.					
By investigators	—	—	1	—	—
By students	—	4	7	—	—

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS IN 1922.

Amherst College.
Barnard College.
Bowdoin College.
Bryn Mawr College.
Butler College.
Carnegie Institution of Washington.
Carnegie Institution, Cold Spring Harbor.
Carnegie Institute of Technology.
College of Physicians and Surgeons.
Columbia University.
Connecticut College.
Cornell University.
Cornell University Medical College.
Dartmouth College.
DePauw University.
Doane College.
Eli Lilly & Co.
Goucher College.
Harvard University.
Harvard University Medical School.
Hunter College.
Illinois Wesleyan University.
Johns Hopkins University.
Johns Hopkins University Medical School.
Knox College.
Lake Forest College.
Massachusetts Institute of Technology.
Mount Holyoke College.
Nela Research Laboratories.
North Carolina College for Women.
Oberlin College.
Princeton University.
Radcliffe College.
Rockefeller Foundation.
Rockefeller Institute for Medical Research.
Rutgers College.
Sophie Newcomb College.
Southwestern College.
Sweet Briar College.
Tufts College.
U. S. Veterans Bureau.
University of Chicago.
University of Cincinnati.
University of Delaware.
University of Illinois.
University of Kansas.
University of Maryland.
University of Michigan.
University of Minnesota.
University of Pennsylvania.
University of the Philippines.
University of Vermont.
University of Wisconsin.
Vassar College.
Wabash College.
Washington University.
Wesleyan University.
Wellesley College.
Western Reserve University.
Wistar Institute of Anatomy and Biology.
Yale University.

Scholarship Tables.

The Lucretia Crocker Scholarships for Teachers in Boston, Since 1888.

Scholarship of $100.00, Supported by a Friend of the Laboratory, Since 1898.

The New London Branch of the American Association of University Women, Since 1920.

5. EVENING LECTURES, 1922.

Friday, June 30,	Dr. Selig Hecht	"The Visibility of the Spectrum."
Friday, July 7,	Dr. H. S. Jennings	"A Critical Study of the So-called Linear Theory of Crossing-Over in Inheritance."
Tuesday, July 11,	Dr. D. H. Dolley	"Specific Functions and Specific Irritability."
Friday, July 14,	Dr. George Sarton	"The History of Science."
Tuesday, July 18,	Dr. E. E. Just	"Certain Effects of Hypertonic Sea-water in Activating *Arbacia* Eggs."
Friday, July 21,	Mr. Wm. Lyman Underwood	"Wild Brother."
Monday, July 24,	Dr. J. A. Detlefsen	"Experiments Regarding the Inheritance of the Effects of Rotation in Rats."
Tuesday, July 25,	Dr. C. B. Bridges	"Chromosomes and Inheritance in *Drosophila melanogaster*."
Friday, July 28,	Dr. G. H. Parker	"Physiology of Actinian Muscle."
Monday, July 31,	Dr. W. H. Weston	"Following a Fungus through the Philippines."
Tuesday, August 1,	Dr. W. H. Lewis	"Tissue Culture."
Friday, August 4,	Dr. J. R. Schramm	"Some Thoughts on Abstracting and Indexing Biological Literature."
Tuesday, August 8,	Dr. W. D. Bancroft	"Structural Colors in Feathers."

6. MEMBERS OF THE CORPORATION.

1. Life Members.

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Carey, Mr. Arthur Astor, Fayerweather St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., Woods Hole, Mass.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
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Mason, Miss Ida M., 1 Walnut St., Boston, Mass.
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Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
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Morgan, Prof. T. H., Columbia University, New York City, N. Y.

Morgan, Mrs. T. H., New York City, N. Y.
Noyes, Miss Eva J.
Nunn, Mr. Lucian L., Telluride, Colo.
Osborn, Prof. Henry F., American Museum of Natural History, New York City, N. Y.
Phillips, Dr. John C., Windy Knob, Wenham, Mass.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Pulsifer, Mr. W. H., Newton Center, Mass.
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Trelease, Prof. William, University of Illinois, Urbana, Ill.
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Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.
Wilson, Prof. W. P., Commercial Museum, Philadelphia, Pa.

2. Regular Members, August, 1922.

Adams, Miss A. E., Mount Holyoke College, South Hadley, Mass.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.
Adolph, Dr. Edward F., University of Pittsburgh, Pittsburgh, Pa.
Agersborg, Dr. H. P. K., University of Nebraska, Lincoln, Neb.
Allee, Dr. W. C., University of Chicago, Chicago, Ill.
Allen, Prof. Ezra, Ursinus College, Collegeville, Pa.
Allyn, Miss Harriet M., Hackett Medical College, Canton, China.
Altenburg, Dr. Edgar, Rice Institute, Houston, Texas.

Anderson, Dr. E. G., College of the City of New York, New York City.

Atterbury, Mrs. Ruth R., College of Physicians and Surgeons, New York City, N. Y.

Baitsell, Dr. George A., Yale University, New Haven, Conn.

Baker, Mrs. L. D., 123 Chiswick Road, Boston, Mass.

Baker, Dr. E. H., 5729 Kimbark Ave., Chicago, Ill.

Baldwin, Dr. F. M., Iowa State College, Ames, Iowa.

Bancroft, Prof. F. W., Aloha Farm, Concord, Calif.

Bascom, Dr. K. F., Allegheny College, Meadville, Pa.

Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, N. Y.

Behre, Dr. Elinor H., Louisiana State University, Baton Rouge, La.

Bigelow, Prof. M. A., Teachers College, Columbia University, New York City, N. Y.

Bigelow, Prof. R. P., Massachusetts Institute of Technology, Cambridge, Mass.

Binford, Prof. Raymond, Guilford College, Guliford College, N. C.

Boring, Dr. Alice M., Wellesley College, Wellesley, Mass.

Box, Miss Cora May, University of Cincinnati, Cincinnati, Ohio.

Bowen, Dr. Robert H., Columbia University, New York City, N. Y.

Bradley, Prof. Harold C., University of Wisconsin, Madison, Wis.

Brailey, Miss Miriam E., Mount Holyoke College, South Hadley, Mass.

Bridges, Dr. Calvin B., Columbia University, New York City, N. Y.

Brooks, Dr. S. C., U. S. Public Health Service, Washington, D. C.

Brumfiel, Dr. Daniel M., University of Iowa, Iowa City, Iowa.

Buckingham, Miss Edith N., 342 Marlboro St., Boston, Mass.

Budington, Prof. R. A., Oberlin College, Oberlin, Ohio.

Bumpus, Dr. H. C., Brown University, Providence, R. I.

Byrnes, Dr. Esther F., 193 Jefferson Ave., Brooklyn, N. Y.

CALKINS, PROF. GARY N., Columbia University, New York City, N. Y.

CALVERT, PROF. PHILIP P., University of Pennsylvania, Philadelphia, Pa.

CARLSON, PROF. A. J., University of Chicago, Chicago, Ill.

CAROTHERS, DR. ELEANOR E., University of Pennsylvania, Philadelphia, Pa.

CARPENTER, PROF. FREDERIC W., Trinity College, Hartford, Conn.

CARROLL, PROF. MITCHEL, Franklin and Marshall College, Lancaster, Pa.

CARVER, PROF. GAIL L., West Lake, Ga.

CASEY, COLONEL THOMAS L., Washington, D. C.

CASTEEL, DR. D. B., University of Texas, Austin, Texas.

CATTELL, PROF. J. MCKEEN, Garrison-on-Hudson, N. Y.

CATTELL, MR. MCKEEN, Harvard Medical School, Boston, Mass.

CHAMBERS, DR. ROBERT, Cornell University Medical College, New York City, N. Y.

CHARLTON, DR. HARRY H., University of Missouri, Columbia, Mo.

CHIDESTER, PROF. F. E., West Virginia University, Morgantown, W. Va.

CHILD, PROF. C. M., University of Chicago, Chicago, Ill.

CLAPP, PROF. CORNELIA M., Mount Holyoke College, South Hadley, Mass.

CLARK, PROF. E. R., University of Georgia, Augusta, Ga.

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COLE, DR. LEON J., College of Agriculture, Madison, Wis.

COLLETT, DR. MARY E., Ashland, Mass.

COLTON, PROF. H. S., Ardmore, Pa.

COOLIDGE, MR. C. A., Ames Building, Boston, Mass.

COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Maine.

COUTANT, MRS. MARY W., Barnard College, Columbia University, New York City, N. Y.

Cowdry, Dr. E. V., Rockefeller Institute, New York City, N. Y.

Crampton, Prof. H. E., Barnard College, Columbia University, New York City, N. Y.

Crane, Mrs. C. R., Woods Hole, Mass.

Curtis, Prof. W. C., University of Missouri, Columbia, Mo.

Curtis, Dr. Maynie R., Crocker Laboratory, Columbia University, New York City, N. Y.

Danchakoff, Dr. Vera, College of Physicians and Surgeons, New York City, N. Y.

Davis, Dr. Donald W., College of William and Mary, Williamsburg, Va.

Davis, Prof. Bradley M., University of Michigan, Ann Arbor, Mich.

Davis, Dr. Alice R., Harvard Medical School, Boston, Mass.

Dawson, Dr. J. A., Dalhousie University, Halifax, Nova Scotia.

Dederer, Dr. Pauline H., Connecticut College, New London, Conn.

Dexter, Dr. J. S., Northwestern College, Naperville, Ill.

Dodds, Prof. G. S., Medical School, University of West Virginia, Morgantown, W. Va.

Donaldson, Prof. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Donaldson, Dr. John C., University of Pittsburgh, School of Medicine, Pittsburgh, Pa.

Drew, Prof. Gilman A., Marine Biological Laboratory, Woods Hole, Mass.

Dungay, Dr. Neil S., Carleton College, Northfield, Minn.

Dunn, Dr. Elizabeth H., Woods Hole, Mass.

Edwards, Dr. D. J., Cornell University Medical College, New York City, N. Y.

Eigenmann, Prof. C. H., University of Indiana, Bloomington, Ind.

Ellis, Dr. F. W., Monson, Mass.

Farnum, Miss Louise W., Peking, China.

Field, Miss Hazel E., Sophie Newcomb College, New Orleans, La.

Finley, Mr. Charles W., Lincoln School, 646 Park Ave., New York City, N. Y.

FISHER, MISS MARY J., Cornell University, Ithaca, N. Y.

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FRY, MR. HENRY J., Columbia University, New York City, N. Y.

GAGE, PROF. S. H., Cornell University, Ithaca, N. Y.

GARREY, PROF. W. E., Tulane University, Richardson Memorial, New Orleans, La.

GATES, PROF. R. RUGGLES, University of London, London, England.

GLASER, PROF. O. C., Amherst College, Amherst, Mass.

GLASER, PROF. R. W., Rockefeller Institute for Medical Research, Princeton, N. J.

GOLDFARB, PROF. A. J., College of the City of New York, New York City, N. Y.

GOODRICH, DR. H. B., Wesleyan University, Middletown, Conn.

GRAVE, PROF. CASWELL, Washington University, St. Louis, Mo.

GRAVE, PROF. B. H., Wabash College, Crawfordsville, Ind.

GREGORY, DR. LOUISE H., Barnard College, Columbia University, New York City, N. Y.

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GUNTHER, MISS MAUDE C., Business High School, Washington, D. C.

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HANCE, DR. ROBERT T., North Dakota Agricultural College, Fargo, N. D.

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HARGITT, PROF. GEORGE T., Syracuse University, Syracuse, N. Y.

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HARRISON, PROF. ROSS G., Yale University, New Haven, Conn.

HARVEY, PROF. E. N., Princeton University, Princeton, N. J.

HARVEY, MRS. E. N., Princeton, N. J.

HAYDEN, MISS MARGARET A., Wellesley College, Wellesley, Mass.

Heath, Prof. Harold, Palo Alto, Calif.

Hegner, Prof. R. W., Johns Hopkins University, Baltimore, Md.

Heilbrunn, Dr. L. V., University of Michigan, Ann Arbor, Mich.

Hess, Prof. Walter N., DePauw University, Greencastle, Ind.

Hinrichs, Miss Marie A., Nela Research Laboratory, Cleveland, Ohio.

Hoadley, Dr. Leigh, University of Chicago, Chicago, Ill.

Hogue, Dr. Mary J., North Carolina College for Women, Greensboro, N. C.

Holmes, Prof. S. J., University of California, Berkeley, Calif.

Hopkins, Dr. Hoyt S., Baylor University, Dallas, Texas.

Hoskins, Mrs. Elmer R., University of Arkansas Medical School, Little Rock, Ark.

Howe, Dr. H. E., National Research Council, Washington, D. C.

Howland, Prof. Ruth B., Sweet Briar College, Sweet Briar, Va.

Hoyt, Dr. William D., Washington and Lee University, Lexington, Va.

Humphrey, Mr. R. R., Cornell University, Ithaca, N. Y.

Hyman, Dr. Libbie H., University of Chicago, Chicago, Ill.

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Kingsley, Prof. J. S., University of Illinois, Urbana, Ill.
Kirkham, Dr. W. B., Springfield College, Springfield, Mass.
Knower, Prof. H. McE., University of Cinncinati, Cincinnati, Ohio.
Knowlton, Prof. F. P., Syracuse University, Syracuse, N. Y.
Kostir, Dr. W. J., Ohio State University, Columbus, Ohio.
Kribs, Dr. Herbert, University of Pennsylvania, Philadelphia, Pa.
Kuyk, Dr. Margaret P., Richmond, Va.
Lancefield, Dr. D. E., Columbia University, New York City, N. Y.
Lange, Dr. Mathilde M., Wheaton College, Norton, Mass.
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Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Md.
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Loeb, Mrs. Leo, St. Louis, Mo.
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Lund, Dr. E. J., University of Minnesota, Minneapolis, Minn.
Luscombe, Mr. W. O., Woods Hole, Mass.
Lyman, Prof. George R., Plant Disease Survey, Department of Agriculture, Washington, D. C.

Lynch, Miss Clara J., Rockefeller Institute, New York City, N. Y.

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McGee, Dr. A. N., Woods Hole, Mass.

McGill, Dr. Caroline, Murray Hospital, Butte, Montana.

McGregor, Dr. J. H., Columbia University, New York City, N. Y.

McIndoo, Dr. N. E., Bureau of Entomology, Washington, D. C.

McMurrich, Prof. J. P., University of Toronto, Toronto, Canada.

Macklin, Dr. Charles C., School of Medicine, Western University, London, Canada.

Malone, Prof. E. F., University of Cincinnati, Cincinnati, Ohio.

Martin, Miss Bertha E., Lindenwood College, St. Charles, Mo.

Mast, Prof. S. O., Johns Hopkins University, Baltimore, Md.

Mathews, Prof. A. P., University of Cincinnati, Cincinnati, Ohio.

Matsui, Prof. K., Imperial College of Agriculture and Dendrology, Morioka, Japan.

Mavor, Prof. James W., Union College, Schenectady, N. Y.

Meigs, Dr. E. B., Dairy Division Experiment Station, Beltsville, Md.

Meigs, Mrs. E. B., 1445 Rhode Island Ave., Washington, D. C.

Medes, Dr. Grace, Vassar College, Poughkeepsie, N. Y.

Metcalf, Prof. M. M., 128 Forest St., Oberlin, Ohio.

Metz, Prof. Charles W., Carnegie Institution of Washington, Cold Spring Harbor, Long Island.

Miner, Dr. Roy W., American Museum of Natural History, New York City, N. Y.

Mitchell, Dr. Philip H., Brown University, Providence, R. I.

Moore, Prof. George T., Missouri Botanical Garden, St. Louis, Mo.

Moore, Dr. Carl R., University of Chicago, Chicago, Ill.
Moore, Prof. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Moore, Dr. A. R., Rutgers College, New Brunswick, N. J.
Morgan, Prof. H. A., Agricultural Experiment Station, Knoxville, Tenn.
Morgan, Dr. Anna H., Mount Holyoke College, South Hadley, Mass.
Morrill, Prof. A. D., Hamilton College, Clinton, N. Y.
Morrill, Prof. C. V., Cornell University Medical College, New York City.
Muller, Dr. H. J., University of Texas, Austin, Texas.
Nabours, Dr. R. K., Kansas State Agricultural College, Manhattan, Kansas.
Nachtrieb, Prof. Henry F., University of Minnesota, Minneapolis, Minn.
Neal, Prof. H. V., Tufts College, Tufts College, Mass.
Newman, Prof. H. H., University of Chicago, Chicago, Ill.
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Osterhout, Prof. W. J. V., Harvard University, Cambridge, Mass.
Packard, Dr. Charles, Union Medical College, Peking, China.
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Payne, Prof. F., University of Indiana, Bloomington, Ind.
Pearl, Prof. Raymond, Johns Hopkins University, Baltimore, Md.

Pearse, Prof. A. S., University of Wisconsin, Madison, Wis.
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Redfield, Dr. Alfred C., Harvard Medical School, Boston, Mass.
Reese, Prof. Albert M., West Virginia University, Morgantown, W. Va.
Reinke, Dr. E. E., Vanderbilt University, Nashville, Tenn.
Rice, Prof. Edward L., Ohio Wesleyan University, Delaware, Ohio.
Richards, Prof. A., University of Oklahoma, Norman, Okla.
Richards, Mrs. A., Norman, Okla.
Rhodes, Prof. Robert C., Emory University, Atlanta Ga.
Robertson, Prof. W. R. B., University of Kansas, Lawrence, Kansas.
Rogers, Prof. Charles G., Oberlin College, Oberlin, Ohio.
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Scott, Prof. G. C., College of the City of New York, New York City, N. Y.
Scott, Prof. John W., University of Wyoming, Laramie, Wyo.
Schrader, Dr. Franz, Bryn Mawr College, Bryn Mawr, Pa.

SHULL, PROF. A. FRANKLIN, University of Michigan, Ann Arbor, Mich.

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SPEIDEL, DR. CARL C., University of Virginia, Charlottesville, Va.

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SEX-DIFFERENTIATION IN THE VIVIPAROUS TELEOST *XIPHOPHORUS HELLERI* HECKEL.

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I. Introduction.

In the fall of 1920, Dr. A. W. Bellamy, who is carrying on breeding experiments with several viviparous teleosts in this laboratory, obtained two *Xiphophorus helleri* from F. E. Boehn, a member of the Chicago Aquarium Club, who claimed that these fish had been subject to sex-inversion. According to this report he had three unusually large specimens—two females and one male—which he kept in a breeding tank and from which he raised more than five hundred young. When the females were about three years old they ceased producing young and, during the course of several weeks, took on the secondary sex characters of males.

When, a few days later, these specimens came into the writer's possession, both were decidedly female as to shape and size of body and decidedly male as to development of anal and caudal fins, which were far advanced toward the male condition yet much belated. Cytological preparations of the entire reproductive system of both fishes disclosed that ripe sperm was in all parts of the duct and gonad, but that the latter was juvenile in comparison to the size and age of the fish.

Suggestive as the case was, the data obtained were far too meager to decide either for or against the reputed transformation. That peculiarities do occur in the sex conditions of these teleosts has been noted in this laboratory as well as by many fish fanciers. The extent and meaning of these peculiarities became the aim of this work. It was thought necessary to approach this problem from three different angles:

1. A detailed study of sex-differentiation in males and females.
2. Isolation of a large number of females for sex observation.
3. An attempt to control sex experimentally.

Only the first part is considered in the present paper. The last two are still in progress and will be the subject of a future publication.

II. Material and Methods.

No attempt was made to study embryonic development during gestation, except for a few later stages, which will be described further on. Development during gestation seems to present some interesting features which will be further investigated as soon as the opportunity presents itself. My material includes fishes from birth to adult stage. To secure all possible stages in sex-differentiation, a large number of fishes were necessary; not less than 400 specimens were studied, of which 300 were sectioned for cytological study.

As to fixatives, Allen's, Bouin's, Child's, Flemming's strong, Smith's, and Zenker's fluids have been used, Bouin's giving constant and satisfactory results. For general stains Heidenhain's hæmotoxylin and alum-cochineal counterstained with orange G were used successfully. For special stains Mallory's triple stain and Harris' hæmotoyxlin were used. All material except adult ovaries were sectioned in paraffin, the latter in celloidin. Very young fishes were sectioned as a whole, in medium-sized fishes the entire viscera were sectioned, and in the mature fishes the gonads and ducts only were preserved for sectioning. For general study sections were cut 6μ in thickness.

The body form of the female is strikingly different from that of the male, which fact can be used to advantage in following the development of the species. To express it in numbers, the length of the fish is divided by its greatest depth. The resulting ratio is known as the form index and can be obtained in two ways, by using either the total length or the body length of the fish as the numerator. The former method is less subject to error in the case of *Xiphophorus helleri* and will be used exclusively in this work.

As the anal fin undergoes a marked post embryonal change in the males in which the third ray increases immensely in diameter, while the fourth and fifth rays, although participating in the transformation, suffer very little, if any, increase in diameter, a ratio of the third by the fourth ray suggests itself. This ratio may be called the fin ratio.

It gives me great pleasure to acknowledge my indebtedness to Prof. F. R. Lillie and Dr. A. W. Bellamy for numerous suggestions and criticisms during the progress of this work. I also wish to express my appreciation for the most painstaking services rendered by our artist, Mr. K. Toda, our technician, Miss D. Brockett, and our librarian, Miss E. L. Dickinson.

III. The Indifferent Stage.

At the time the young fishes are born they measure on the average 8 mm. in total length, while sex is unmistakably established at the length of 10 mm. This means that the indifferent stage of sex development is almost entirely pre-natal. The form index is 6.26 and the fin ratio is 1.0 at birth. The gonads, although small, are distinctly set off from the surrounding tissues and lie one on each side of the body cavity, immediately below the air bladder, suspended in a peritoneal sac. The peritoneal sac, which becomes the permanent wall of the gonad, is a protruded portion of the lining of the body cavity. The gonad inside of this sac consists of two kinds of cells:

1. Primordial germ cells.
2. Very much smaller cells with elongated nuclei, which can not be distinguished from the peritoneal cells and which tend to surround the germ cells to form follicles (Fig. 1).

The primordial germ cells are unmistakable. They are the largest cells in the body of the fish and measure on the average, cell 14.4 μ, nucleus 8.4 μ, in diameter. The nucleus takes a lighter stain than the cytoplasma and possesses one more or less distinct nucleolus. The chromatin occurs most commonly in strands or loops immediately beneath the nuclear membrane. The germ cells may be single or in nests of two or more cells.

As stated above, the pre-natal developmental changes have not been studied systematically. Several stages, however, are on hand. The gonads assume the bilateral position at about 3.2 mm. in total length. Before that the germ cells are in a single mass placed medially in the body cavity. The peritoneum already surrounds the gonad more or less loosely and here the mingling of the peritoneal cells with the germ cells is very evident (Fig. 3). As these rather small insignificant cells, mingling with the primordial germ cells, play by no means an insignificant rôle in later differentiation, I have been particularly anxious to trace their origin, and must conclude that no criterion whatever separates them from peritoneal cells. This view is consonant with that of McLeod (1881), Jungerson (1889), and Eigenmann (1897). The primordial germ cells of the 3.2 mm. stage are very conspicuous structures. The cell is 11.8 μ and the nucleus is 6.4 μ in diameter. The nucleus may be lobulated and appear as if more than one nucleus was present in a cell (Fig. 3). The cytoplasm is yolk laden. In short, the conditions of these cells indicate an early segregation.

IV. Early Sex-differentiation of the Female.

The earliest stage of the ovary shows very little difference from the indifferent gonad. In the early development of the ovary the primordial germ cells grow in size and form follicles. At the time these follicles have reached approximately the medium size they degenerate and are absorbed. This process is referred to as retrogression. Definitive germ cells originate from peritoneal derivatives later and differentiate into functional follicles of the adult ovary. All young females undergo retrogression, of which three classes may be distinguished. In some, hereafter distinguished as class 1, formation of definitive follicles begins before retrogression is well advanced. These comprise about 50 per cent. of all young females (see Table IV., p. 68). In others (class 2) no formation of definitive follicles precedes complete degeneration of primordial follicles, but indifferent germ cells are seen in the epithelium before degeneration is complete. In others, again (class 3), retrogression of primordial germ cells is complete before any germ cells appear in the epithelium. In the first class there is no doubt that development into functional females occurs; the later history of the other two classes is considered beyond. The ovarian cavity and the oviduct are formed comparatively early in the development of the ovary.

1. *Early Normal Ovary.*

The total length of the young female varies from 9.3 to 16.9 mm. The average form index is 5.02 and the fin ratio is 1.13.

The indifferent gonad passes into the ovary very gradually. Paired and considerably apart at the beginning, the two ovaries approach each other until they meet medially and fuse into one gonad which is the normal condition of the adult ovary. The external contour of the gonad is very regular and its component cells, the primordial germ cells and the peritoneal cells, are evenly distributed (Fig. 2). The gonad gradually increases in size owing to the multiplication of its component cells, although no mitotic figures have been discovered. This is true of all the material on hand.

At the time the two gonads have approached each other the picture has changed considerably. The primordial germ cells increase in size and become completely invested by peritoneal cells to form a follicle of one layer of cells thick. The growth of the germ cells is not simultaneous, some grow faster than others, but all the germ cells present in the gonad are subject to a transformation to young ova at this stage or the early phase of the next stage. Blood vessels which are present in the mesentery enter the young ovary in the form of capillaries.

The shape of the approaching gonads changes. Instead of being broader laterally they now deepen dorso-ventrally. Fusion of the two gonads take place anterio-posteriorly, not along the entire surface of contact, but on the dorsal and ventral margins only. The space between becomes the ovarian cavity (Figs. 4–5). It will be recalled that the external lining of the gonad is of peritoneal origin, which means that the epithelium of the ovarian cavity is of peritoneal origin. At first the ovarian cavity is a narrow slit with its larger axis running dorso-ventrally; very soon, however, this picture changes to a cross-shaped lumen produced by the invagination of the epithelium laterally. This form of the lumen persists for a considerable time in the young ovary, but ultimately becomes very much modified until in the adult ovary no trace of the original form remains (Fig. 27). That portion of the mesentery which comes to lie between the fusing gonads becomes absorbed. The dorsal portion, or mesovarium, attaches the ovary to the body wall dorsally, while the ventral portion connects the gonad with the rectum ventrally.

The formation of the oviduct is begun at the time the two gonads are in close proximity. By the time the gonads are completely fused the duct tissue extends from ovary to urogenital sinus with complete lumen at anterior and posterior ends, but none or only a beginning centrally. In fact, the lumen of the oviduct appears first in its posterior terminal, and only later, at the anterior, end. This, of course, would indicate that the duct formation proceeds from two primordia, one anterior and the other posterior, which is the case in *Xiphophorus helleri*. The anterior primordium is the posterior end of the ovary and the posterior is the lining of the body cavity at its most posterior end in the region of the urogenital sinus. Before there is any indication of duct formation anteriorly, the posterior primordium is actively proliferating cells which at first form a solid cord along the median portion of the peritoneal lining of the body cavity on the dorsal edge of the mesentery. This cord extends anteriorly and at the time a similar strand of cells is formed from the anterior primordium a lumen has appeared in the former posteriorly (Figs. 10–11). No liquefaction of cells has been noticed in the formation of the lumen.

The anterior cord of cells is a direct extension of the posterior ventral portion of the ovary. As that part of the ovary has not as yet fused dorsally, the extending cord is V-shaped with the apex ventrally (Fig. 6). This portion of the duct grows posteriorly on the dorsal edge of the mesentery until it reaches and fuses with its posterior member. The mesovarium thickens and flattens above the posterior portion of the ovary and finally comes in contact with the open end of the V to fuse and thus form the lumen in the oviduct from the anterior primordium (Figs. 6–8).

That the duct starts to grow from two sources was first noticed in an abnormality in which the two growing tips overlapped with the urinary bladder between. In normal development the duct forms ventral to the urinary system, but in the case in question the anterior part of the duct had pushed above the urinary bladder, while that from the posterior end was in the normal path. The overlapping was for a considerable distance. This stimulated closer study, and it was found that at the time when cords are formed at both ends no traces of such exist centrally (Fig. 14), nothing but a single layer of peritoneal lining. Further it was ascertained that by far the larger part of the duct comes from the posterior source.

In the primordial germ cells of the indifferent stage there is only one nucleolus with irregular, generally spherical contour. It always stains with chromatin dyes. Later its contour becomes perfectly regular and it enlarges. Still later two nucleoli are usually found, though as many as six may occur. In the ova of medium size (.272 mm.) the nucleoli are conspicuous bodies and may measure 10 μ in diameter. A body similar to the nucleolus in stain and size was noticed in the cytoplasm. At first it was taken for a centrosome, but as it showed no structure whatever the first hypothesis had to be abandoned. Subsequently it was noticed that one of the two nucleoli moves toward the nuclear membrane and passes through it (Fig. 13). When it reaches the cytoplasm it does not stay there, but moves toward the follicular wall (Fig. 16). It is probable that the migrating nucleolus passes out of the follicle, as it can be seen in all parts of the cytoplasm. The actual process of passing through the follicular wall has not been noticed, but bodies which answer to the description of the nucleolus are occasionally encountered between the follicles. Such migrating nucleoli are by no means uncommon, as many as six have been noticed in one young ovary. The direction of migration is not predetermined. In retrogressive ova its shape is greatly affected. It may be elongated, crescent-shaped, irregular, or hollow.

2. *Retrogression in Class 1.*

The size limits of the fish vary from 16.5 to 29.5 mm. in total length. The form index averages 4.62 and the fin ratio is 1.16. The germ cells all derived from primordial ova in this stage are anywhere in size between primordial germ cells and medium-sized ova. However, the larger-sized ova are affected first. The first perceivable sign of retrogression appears to be a darker staining zone in the cytoplasm surrounding the nucleus. Later this zone gradually disappears, but at the same time the cytoplasm loses its affinity for stain and appears bleached. The follicular wall, which is one layer of cells thick in the normal ova, now becomes disorganized and it may form two or more layers of cells which may form strands and migrate into the yolk (Fig. 15). The nucleus appears to be affected last. It shrinks, becomes irregular, and disintegrates together with the yolk mass. After the ovum has been entirely absorbed remnants of the follicle remaining for some time witness to the destruction. Some such atrophied follicles may contain several isolated cells which no doubt are the remains of the ingrowing follicular strands. Sooner or later these remnants are also resorbed (Fig. 25).

The retrogression is further characterized by the fact that the epithelium of the ovarian cavity shows no signs of being unfavorably affected. On the contrary it appears very active. It has been noticed, particularly when the ova are greatly reduced by disintegration, that new primordial germ cells originate in the proximity of the epithelium of the ovarian cavity. These could not be traced to cell division, nor could they be traced to preexisting smaller primordial germ cells; the source of these cells is the epithelium itself.

It appears probable that any cell in the epithelium is capable of transforming into a germ cell. All stages of such transformation are encountered in the epithelium as will become apparent from Figs. 17–24. The epithelial cells are small with relatively little cytoplasm; the nucleus is elongated and may be more or less irregular in contour. The chromatin is granulated and an ill-defined nucleolus can occasionally be noticed. The nuclei take a dark stain and contrast strikingly with the germ cell nuclei. The first sign of such metamorphosis appears to be the activity of the chromatin in which the granules become coarser, forming more or less into lumps. This phenomenon is closely followed by the growth of nucleus and cytoplasm. The elongated and irregular contour becomes regular and the stain loses its density. The final product of such transformed epithelial cells can not in any way be distinguished from primordial germ cells (Figs. 1–2). As these cells increase in size they passively move from the epithelium into the cortex of the ovary to develop into follicles. Fully formed germ cells do occur in the epithelium, which will be discussed later. The transformation of epithelial cells into germ cells is by no means a rarity. It is a common occurrence in all phases of ovarian activity of the females belonging to class 1. Occasionally a place in the epithelium may be found where all stages of such transformation occur in the same field of the microscope. Such a condition is found in Fig. 12. At least six definite stages (*a–f*) are shown in the figure which range from epithelial to completely formed germ cells.

It will be remembered that the epithelium of the ovarian cavity originates from the peritoneal lining of the fusing gonads, and that no germ cells whatever enter into its composition at any time.

The formation of germ cells from epithelium of the ovarian cavity is by no means confined to *Xiphophorus helleri* among the teleosts. Similar observations have been made by Hoffmann (1886) and Böhi (1904) on salmon, Wallace (1904) on *Zoarces viviporus,* Philippi (1908) on *Glandichthys januarius, et al.*

After the transformation process of the epithelium cells into germ cells was discovered, it became apparent that such transformation may occur at any place in the ovary directly from the free cells of peritoneal origin (Fig. 15, *tpc*). The process is the same in principle as described above.

3. *Retrogression in Class 2.*

The general appearance of females of this class is not materially different from class 1. The total length varies between 14.2 and 29.6 mm. The form index averages 4.53 and the fin ratio is 1.17. There are generally fewer primordial ova in the ovary than in the previous class and hardly any one of them appear normal. The most important characteristic of this class lies in the fact that the epithelium of the ovarian cavity is relatively inactive.

The epithelium of the ovarian cavity is no longer surrounded by germ cells, nor are there many at any other place. Even the stroma is very sparingly represented. The ovary looks empty. The outer ovarian epithelium, although intact, shows similar signs of inactivity. Blood vessels are the most prominent structures in the ovary in this class of disintegration.(Fig. 26).

4. *Retrogression in Class 3.*

The size limits in this class vary from 18.4–65.0 mm. in total length., The average form index is 4.43 and the fin ratio is 1.84. From Table I. it will be seen that the form index of this class is much closer to that of the male than to that of the female, and that the fin ratio is decidedly that of a young male. In fact, in some of the specimens in this class the anal fin has advanced considerably in the process of transformation. These facts are evidently significant and they will be referred to in connection with the differentiation of the male sex.

Conditions are not less significant as regards the ovary. The outer epithelium of the ovary has been either completely resorbed or else is in advanced stages of resorption. Ova of all stages have left nothing but inconspicuous traces of former presence. Nothing but the epithelium of the ovarian cavity and the oviduct has withstood the destructive process (Fig. 28). The epithelium has shrunk considerably and is by no means of the same appearance in all cases. It looks inactive in some and active in others, although proliferation of germ cells is not apparent. Judging from the appearance of the ovarian remains, it can be said that the renewed activity of the epithelium in later stages of retrogression is not simultaneous with degeneration, but begins afterwards. Remains of degenerating follicles may be found in the body cavity. Occasionally such remains may assume activity and form cords of various sizes, which may persist for a considerable time.

The fatty tissue which commonly surrounds the gonads in fishes increases immensely during the retrogression. In fact, in the later phases of disintegration of the ovary special care must be taken to discover the remains of the ovary within the fatty mass. The fact that it contains an abundance of large blood vessels leads one to suspect that it might play some rôle in the disintegration process. Although stroma cells have been noticed among the fatty cells, at no time do the latter engulf, surround, or even come in contact with the disintegrating ova. Whatever physiological rôle they play as regards the fate of the germ cells can not be discovered by observation. Experimentally it has been noticed that the fatty tissue increases immensely within two days after injury to the reproductive system.

TABLE I.

Stages in Development.	Averaged Data.		Limits.
	Form Index.	Fin Ratio.	Total Length of Fish in Mm.
Indifferent	6.26	1.0	0 – 9.3
Normal Immature Females	5.02	1.13	9.3 – 16.9
Retrogression in Class 1	4.62	1.16	16.9 – 29.5
Retrogression in Class 2	4.53	1.17	14.2 – 29.6
Retrogression in Class 3	4.43	1.84	18.4 – 65.0
Normal Mature Females	5.34	1.28	26.7 – 80.0
Early Tubule Formation	5.17	1.16	9.3 – 13.5
Middle " "	4.84	1.27	12.5 – 18.6
Late " "	4.64	1.74	15.7 – 51.5
Mature Males	4.20	4.25	31.6 – 84.4

V. EARLY SEX-DIFFERENTIATION OF THE MALE.

The testis of *Xiphophorus helleri* belongs to the acinus type in which seminiferous tubule formation is greatly modified. Sex-cords and tubules are formed from peritoneal cells exclusively. Such tubules give rise to the acini by transformation of epithelial cells into germ cells. The early sex-differentiation of the male is principally a process of tubule formation, and thus it may be divided into early, middle, and late stages of tubule formation.

1. *Early Stage of Tubule Formation.*

The size limits of the young male vary from 9.3 to 13.5 mm. in total length. The average form index is 5.17 and the fin ratio is 1.16, which differs very little, if any, from that of the female in similar stage of development.

The gonads, like those of the female, are far apart at the beginning of sex-differentiation. Gradual approach follows until they meet medially below the air bladder to be separated only by the mesentery. Unlike the ovary, the testes remain permanently paired. The mesentery is well supplied with blood vessels, which at this stage supply the gonads also. There is no difficulty whatever in distinguishing the testis from the indifferent gonad even in early phases of sex-differentiation. It will be remembered that such difficulty does exist in the case of the female. A comparison of Figs. 1 and 2 will make this clear. Even in much earlier stages (Fig. 3) this similarity exists. It may be that the indifferent stage is an early female stage.

Although I have not counted the germ cells in various stages of development, it is nevertheless apparent that the number of the primordial germ cells in the young testis is appreciably less than in the young ovary. Germ cell division was not seen and the cells usually remain isolated. The important rôle in the differentiation of the male is not played by the primordial germ cells, but by the peritoneal cells. The latter proliferate abundantly, and instead of being evenly mingled with the primordial germ cells, cause a segregation of the latter at the periphery of the testis, while the peritoneal cells occupy the center and inner margin (Fig. 30). This aggregation may be known as the sex-cord and, as will be seen in the next stage, is a preparation for tubule formation. In this stage also some of the peritoneal cells are investing the primordial germ cells. Such activity would indicate follicle formation, which is not an adult male structure.

2. *Middle Stage of Tubule Formation.*

The size limits of animals in this stage vary from 12.5 to 18.6 mm. in total length. The form index is 4.84 and the fin ratio is 1.27.

The primordial germ cells play no perceptible rôle in this stage.

They remain on the outer periphery of the gonads, apparently inactive. All activity is vested in the cells derived from the peritoneum. These cells form at first a solid cord, the sex-cord, in the central part of each gonad, parallel with the longer axis of the animal. The cord formation in the young testis is antero-posterior, but as the elongation of the gonad is mostly anteriorly, the cord formation later follows the elongation of the testis. The cord resembles very much a tubular gland except that there is no lumen at first. The peritoneal cells place themselves side by side with their apices pointing centrally in the sex-cord, which in a transverse section appears rosette-like (Fig. 31). These cells increase considerably in size and number, which causes the increase in diameter of the cord and the origin of a central lumen; or, in other words, transforms the cord into a tube which is the testicular cavity or sperm duct. It must be pointed out that there is no indication whatever that any of the germ cells participate in the formation of the sperm duct.

3. *Late Stage of Tubule Formation.*

The size limits of animals in this stage vary from 15.7 to 51.5 mm. in total length. The wide range is significant in that it shows great variation in the time of sex-differentiation in the male. The average form index of this period is 4.66 and the fin ratio is 1.74, which is decidedly male. There is a slight increase in diameter in the third ray in the preceding stage, but, strictly speaking, transformation of the anal fin into an intromittent organ or gonopod does not begin before the present stage is reached. The gonad is decidedly differentiated as testis before the anal fin is affected. The first indication of the transformation of the anal fin into a gonopod is the thickening of the third ray. This is shortly followed by the elongation of the third, fourth, and fifth rays until approximately twice the length of the original fin is reached. The rest of the rays—*i.e.,* the first, second, and seventh to eleventh—are not subject to any particular change.

The gonad has increased appreciably in size owing primarily to the proliferation of the peritoneal derivatives and only secondarily to increase of the primordial germ cells. Most commonly the testis is butterfly-shaped in the transverse section, but may be very irregular, depending upon its previous history. The primordial germ cells are almost invariably in nests, which would indicate that cell division has taken place; mitotic figures, however, have not been seen. These nests of germ cells always occupy the periphery of the testis and have no connection with the tubules.

The sperm duct or the main tube of the testis of the previous stage is now branched and as development proceeds the branches become subdivided (Figs. 32 and 38–39). Each testis may be roughly compared to a bunch of grapes with the sperm duct as the main stem. The tubules thus produced are radially arranged with their apices toward the periphery of the gonads. This tubule formation is peculiar to *Xiphophorus helleri* and closely related genera and, as far as the writer knows, has not been described before.

If one of these radial tubules is examined, it will be found that it consists of an inner epithelial layer and an outer homogeneous membrana propria (Fig. 33). This membrane is very thin and contains a very few small nuclei. The epithelial cells are cuboidal to columnar in shape, according to stage of development. In size and staining reaction the cells are intermediate between peritoneal and primordial germ cells. In more advanced stages of development the tubules show various stages of germ cell metamorphosis (Figs. 33–36). The apex may become separated from the rest of the tubule by a constriction and assume a spherical form; the tubular lumen has become obliterated by the growth of cells which are now spherical in shape and differ from the primordial germ cells at the periphery of the testis only in position. The next portion of the tubule shows cells in a less advanced stage; the lumen may still be present, the cells and particularly the nuclei are smaller in size and less regular in shape; the cells take a darker stain than the apical cells and there is no delimiting membrane from the more basal cells. The proximal portion of the tubule may differ in no essentials from the young tubule except that the cells show an increase in size. Later on the acini separate completely from the tubule, but for a long time the radial arrangement is maintained (Figs. 37–38).

It will be remembered that in the ovary definitive germ cells are formed from two different sources:

1. The epithelium of the ovarian cavity.

2. Free cells in the ovarian cortex of peritoneal origin. Similar conditions are found in the testis. The first and by far the most common source is the epithelium of the tubules described above. The second source, precisely as in the female, is the free peritoneal cells of the testicle. The method of transformation differs in no essentials from that found in the ovary. Such germ cells move toward the periphery of the gonad, multiply, and eventually become spermatocysts.

The variability in the development of the testis in this stage can not be overlooked. They occur too often and too regularly to be classified as haphazard abnormalities. The testis of the young fish from 15–20 mm. in total length is a very definite structure. Its definite shape, its regular contour, and its distinct bilaterality are characteristic (Fig. 38). In cases where transformation occurs, however, the gonad varies in shape, is irregular in contour, and, what is more striking, is only partially bilateral. Generally the posterior part of the testis shows more or less complete bilaterality, while the anterior part is in a fused state, thus producing a bifurcated testis (Figs. 40 and 41). There is no indication whatever that the typical shape of the testis occurring in those specimens that transform early becomes irregular and the bilateral testis becomes fused as development advances. On the contrary, the typical testis maintains its definite shape during the life of the animal, while the irregularly shaped testis gradually becomes regular as development proceeds, and at the time of spermatogenesis the shape of the testis is more or less definite in all. All of the above facts lead to the inference that the bifurcated testis has resulted from the epithelium of the ovarian cavity after complete disintegration of the ovules. In fact, all stages can be found between the epithelium of a degenerated ovary and a testis.

The formation of the extra-testicular sperm duct belongs to this stage, although some traces of it are already found in early and middle stages of tubule formation. Generally speaking, the male and female ducts are homologous. Both begin from an anterior and a posterior primordium. In both the posterior primordium is more advanced in origin and extent of formation than the anterior primordium. In both the anterior portion of the duct is of peritoneal origin, and this is in all probability true of the posterior portion as well. The formation of the lumen of the posterior part of the duct is the same in both sexes. The anterior portion, however, presents some slight differences. In the male the lumen is formed by the extension of the intra-testicular sperm duct into the posterior primordium. The ends of the two sperm ducts meet slightly back of the testis and fuse to form a single duct. Not only is the origin and method of formation of the male and female ducts similar, but the young sperm duct and the young oviduct are identical in structure. Specialization takes place only later in development.

VI. Late Sex-differentiation of the Female.

The smallest normal mature female found in my material is 26.7 mm. in total length. The largest is 80.0 mm. long, which can be considered a full-grown specimen. The average form index is 5.34 and the fin ratio is 1.28.

Externally the sexually mature female can be recognized by the appearance of a dark spot on each side in the region of the pelvic fins. This is not due to pigmentation of the dermis, but of the peritoneal lining of the body cavity above and posterior to the ovary. In males and young females the peritoneal lining is silvery white or slightly pigmented. As the female approaches sexual maturity the pigment increases immensely and can be seen through the translucent body wall as a black spot. The name "Trächtigkeitsfleck" has been applied to denote this characteristic, but since it is maturity instead of "Trachtigkeit" that causes its appearance, a more appropriate name would be puberty spot. The size of the spot varies directly with the size of the ovary and the period of gestation. It is very conspicuous at the time of the birth of the young and this probably explains the origin of the German name.

A female with a puberty spot always contains mature ova. There may be from one to nearly one hundred in number, depending upon the age of the fish. They are of a brilliant amber color, measuring on the average 1.6 mm. in diameter. The ova lie in the cortex of the ovary and often occupy the entire space between ovarian lumen and ovarian wall (Fig. 27). Each is contained in a follicle consisting of a single layer of cuboidal cells. The follicles are surrounded by a very slightly developed theca folliculi which emerges indistinctly into the surrounding stroma. Immature ova of all sizes can be found all over the ovary, but most commonly in the region of the epithelium of the ovarian cavity. Yolk is deposited at the time the ova have reached medium size. At this time the young egg has a beautiful alveolar structure in section due to the presence of oil globules which have been dissolved by reagents.

The epithelium of the ovarian cavity of the adult *Xiphophorus helleri* differs in no essentials from that of *Glaridichthys* described by Philippi (1908). Owing to the encroachment of the adult ova it has been thrown into numerous folds which occasionally are not unlike the villi of the small intestine. The characteristic depressions in the epithelium of the ovarian cavity called "Delle" by Stuhlmann (1887) are very prominent in *Xiphophorus helleri*. Each depression is an invagination of the epithelium of the ovarian cavity into the ovarian substance directed toward an egg. It serves two purposes: admission of the sperm into the ovum and creation of a place of rupture for the escaping young. Its formation begins with rather young ova, slightly below medium size, but is not completed until the time of fertilization.

The oviduct of the adult female is very short. It consists of three layers: an outer muscular, a middle connective tissue, and an inner epithelial. The latter is thrown into folds which may either project into the lumen like villi or overlap and form pockets. According to Philippi (1908) these pockets serve as hiding places for spermatozoa in which they may remain and maintain vitality for over 160 days. The oviduct enters the uro-genital sinus at its anterior border, projects backward to open immediately in front of the aperture which is located slightly back of the anus.

VII. Late Sex-differentiation of the Male.

Here are comprised the stages in which spermatogenesis takes place. The size limits vary from 30 to 84.4 mm. in total length. The form index of the sexually mature male averages about 4.2, while the fin ratio is 4.25.

The various stages of spermatogenesis are rather difficult to describe owing to the fact that cell division has thus far evaded the writer's observation. Of course, the difficulty lies mostly in the proper application of terminology and not so much in the changes that take place.

As has been pointed out, the acinus or spermatocyst is the end product of the tubule formation and the beginning of spermatogenesis. The definitive germ cells in the acini approach the size and appearance of the primordial germ cells before spermatogenesis begins. At this period the nuclei of these cells measure from 7.2 to 9 μ in diameter. This is the largest size they ever reach. The cell dimensions are hard to measure because the entire acinus presents the aspect of a syncytium (Fig. 36). On account of the size and what is to follow, it would seem proper to term these cells primary spermatocytes. The next thing that happens to the acini is the doubling of their volume and the number of their cells. Evidently cell division has taken place. The picture has changed radically (Fig. 37, *ssc*). The nuclei no longer show a clear aspect with more or less thread-like chromatin; the stain is darker, the chromatin is broken up into a great many rod-like structures; the nuclear membrane is absent and the cytoplasm is greatly diminished. The nuclei now measure on the average 5.96 μ in diameter. This might be considered a secondary spermatocyte stage.

Apparently another division takes place as the number of nuclei doubles. This time there is no appreciable increase in size of the acini. The size of the nucleus, however, has diminished to 2.5 μ, which is practically one half of the previous stage. The color has not changed, but the cytoplasm is reduced to a minimum; in fact, it can hardly be demonstrated with general methods of staining (Fig. 37, *sds*). It is probable that we are dealing with the spermatid stage.

In the next stage the metamorphosis of the spermatids into spermatozoa is quite evident. For the first time the acinus becomes luminated as the developing spermatozoa move toward the very thin, homogeneous wall of the acinus, where they form a complete layer of one layer of cells thick. The tail differentiates and the spermatozoa are all oriented so that the tail is free in the lumen. The head, or rather the nucleus, gradually decreases in size as it elongates. The final dimensions are 1.35 x 2.55. There is also a decrease of size in the entire acinus or spermatophore, which measures about 50 μ in diameter, or half that of the secondary spermatocyte stage. The heads of the spermatozoa are so closely pressed together that they appear like a single layer of epithelial cells (Fig.

37, *sph*). They stain very dark and with iron-hemotoxylin the cross-section of a spermatophore presents a solid dark ring. The tails are fairly long and are twisted altogether in a sort of spiral. Occasionally a belated spermatozoan has failed to get "in line" and remains in the lumen among the tails. The ripe spermatophores occupy the central portion of the testis and are found in every part of the sperm duct. In this condition they are discharged and reach the female genital tract, where by the action of the ovarian secretion the outer membrane is dissolved and the spermatozoa swim freely in the oviduct or ovarian cavity.

The question naturally arises as to the meaning of the primordial germ cells at the periphery of the testis (Figs. 37–38, *ppgc*). In comparison with the rest of the germ cells, they appear inactive. It can not be said with certainty that their number decreases with age, nor is there any reliable sign that they take part in spermatogenesis. In a few diseased males which were affected by a tumor growth in the tail region the testis was affected to a marked degree. All the spermatophores, mature and immature, were broken up with the spermatozoa free in the lumen. Formation of new acini was going on in all parts of the testis, but the peripheral primordial germ cells showed no more activity than in the normal. This seems to lend evidence that the primordial germ cells do not furnish a source of definitive germ cells.

It seems to be necessary to emphasize at this place that no retrogressive development of any sort is encountered in the normal process of sex-differentiation in the male of *Xiphophorus helleri*.

The sperm duct is slightly longer than the oviduct because the testis is more anterior in position. It consists of three layers: an outer muscular, a middle connective tissue, and an inner epithelial. The cells of the epithelium are flagellated. The contour of the epithelium is very regular. Just as in the case of the female, the sperm duct enters into the uro-genital sinus to open just in front of the aperture. In both male and female the urethra opens into the anterior part of the uro-genital sinus. The uro-genital aperture lies at the base of the gonopod and does not enter into it.

It will be remembered that the transformation of the anal fin into an intromittent organ or gonopod begins during the late stage of tubule formation. The first noticeable thing in such metamorphosis is the thickening of the third ray; in fact, it should be said rays, as all of them are paired and lie side by side (Figs. 44 and 45). As the thickening of this ray continues, the third, fourth, and fifth rays elongate until approximately twice the length of the original fin is reached (Figs. 48 and 49). Next the tips of the last-named rays form knob-like projections which are to be transformed into hooks in later development (Fig. 47). As a general rule, spermatogenesis begins at this stage of the transformation of the anal fin, although variations are not uncommon. The first, second, and sixth to tenth rays are subject to no special changes and remain rudimentary. The third ray, which has increased immensely in thickness medially, forms an S-like structure apically and the distal arm projects out on the ventral margin of the fin to form a copulatory hook. This hook is reinforced by the tip of one of the members of the fourth rays, which has turned ventrally, and we shall call it the ventral part of the fourth ray. The same kind of a hook is formed on the opposite margin of the fin by the union of the two members of the fifth ray (Fig. 46). Furthermore, the third and the dorsal part of the fourth ray form two rows of symmetrically placed "teeth" which project backwards and outwards, forming a hollow external groove in the former which leads toward the tip of the gonopod. There are approximately 8 "teeth" on each side of the third ray and 9 in the fourth. The two members of the fifth ray in the secondary growth regions, anterior to the hook, have fused and broadened latero-dorsally to form a concave groove on the dorsal margin of the gonopod. All the apical modifications of the rays are supposed to be for copulation and transmission of the spermatophores from the male to the female. At the time the anal fin is metamorphosed into a gonopod the spermatophores are formed and ready for discharge.

Besides the gonopod the beautiful sword of the *helleri* male and the pelvic fins are secondary sex characters and must be considered here. Both of them, however, start their transformation somewhat later than the anal fins.

The sword shows the first signs of development shortly before spermatogenesis begins. It is formed from the ventral lobe of the tail fin of the male fish. The ten ventral rays of the caudal fin are involved; most of the elongation falls upon the sixth to tenth and the utmost length is reached by the eighth ray. In the adult male the length of the sword approximates the total length of the fish. The pigmentation of the sword is very striking. The middle rays are from greenish to orange in color; this is bounded on both sides by deep black. The dorsal portion of the caudal fin has a yellowish hue.

The pelvic fins of the male differ from those of the female in the relative length of the rays. The length of the first or anterior ray of the pelvic fin of the male is to the second ray as 1 : 2, while the reverse is true of the female—*i.e.*, as 2 : 1. These differences appear late in sex-differentiation, approximately at the beginning of spermatogenesis.

VIII. Sex-ratios.

The sex-ratio in the immature fishes between the sizes of 10–26 mm. in total length is given in Table II. These limits were chosen because sex is unmistakably established at 10 mm. and normal adult females appear at about 27 mm. in total length. The specimens in this table were taken at random and thus they present the actual conditions in the population.

Table II.

Normal Females.		Retrogressive Females.		Males.		Total.		Percentage.	
No.	Size.	No.	Size.	No.	Size.	Males.	Females.	Males.	Females.
21	10 – 16.7	58	12.5 – 26	44	10 – 26	44	79	36%	74%

It is very clear from the above table that there is a great preponderance of females in *Xiphophorus helleri* in the immature condition—*i.e.*, in the developmental stages falling between 10 and 25 mm. in total length. For all practical purposes the ratio may be reduced to two females to one male.

For sex-ratios in mature fishes the writer is indebted to Dr. A. W. Bellamy. Table III. is compiled from his data. Each record gives the history of an entire brood reared from birth to sexual maturity.

TABLE III.

Record.	No. Born.	Females.	Males.	Unaccounted.	Percent. of Males.
106 *E*	40	1	35	4	94
114 *A*	13	2	11	0	85
106 *D*	81	0	60	21	100
112 *DEF*	106	26	33	47	56
112 *G*	47	27	6	14	18
34 *AB*	12	1	9	2	90
113 *ABC*	17	2	10	5	83
Total	316	59	164	93	75%

From the above tables it is evident that the sex-ratios have suffered reversal from immature to mature conditions; also that the change is very marked. One can perceive that such change could be brought about in two ways: differential viability and sex inversion.

Oxygen consumption experiments have shown (Bellamy, 1922) that the males consume approximately twice as much oxygen as the females. The males are also by far the more active. From these data one might expect to find greater mortality among the males than among the females. This is borne out experimentally. If both sexes of equal chronological age are subject to unfavorable conditions such as weak solutions of potassium cyanide, alcohol, or excesses of temperature, the male invariably succumbs first. With some of these conditions, such as cyanide or alcohol, the females live twice as long on the average as the males and sometimes even longer. That the reversal of the sex-ratio is not due to mortality of the females can also be ascertained by keeping strict records of broods of fishes from birth to sexual maturity. The mortality of *Xiphophorus helleri* under proper conditions is slight, indeed, and whenever death occurs the sex can be established cytologically. The results of such observations are decidedly in consonance with the experimental results and it is perfectly safe to conclude that the female is at least as sturdy as the male.

If the reversal of sex-ratios from immature to mature condition is not due to differential viability, there is only one other possibility, and that is sex inversion. This occurs most commonly in fishes from 16–27 mm. in total length, but may occur at any size from 16 mm. upward.

IX. Inversion of Females.

It will be seen in Table II. that all females between 16.7 and 26 mm. are retrogressive. Normal females do not occur before they reach approximately 27 mm. in total length. The phenomenon of retrogression in *Xiphophorus helleri* is not a seasonal fluctuation, as this species produces young practically every month. Nor is it temporary and reversible, once the ova are affected they can not be rejuvenated. Lane (1909) has described retrogressive ovules in *Lucifuga* and *Stygicola.* In these teleosts the larger and more favorably situated ova cause the disintegration of smaller ovules which are absorbed as food. It is very clear that this is not the case in *Xiphophorus,* for it is the larger ova that are affected first. These may be in a hopeless state of degeneration before the smaller ones show signs of disintegration. My material strongly favors the conclusion that all of the linear descendants of the primordial germ cells disintegrate.

If the retrogressive females transform into males, it is apparent from the above tables on sex-ratios that approximately half of the females become males. It is doubtful whether any of the females of class 1 transform, because the ovarian epithelium is actively proliferating definitive germ cells. The activity of the epithelium is relatively slight in females of class 2 and stops entirely in females of class 3, and therefore one would expect that the prospective males are recruited from the two latter classes. If so, the sum of the females of classes 2 and 3 must approximately equal the sum of females of class 1. This is actually the case, as is evidenced by the following table, in which all of the retrogressive females studied are tabulated.

Table IV.

Class 1.	Class 2.	Class 3.
40	25	10

The question may be raised whether the retrogressive females result in sexual forms. In the first place, no sexless form has been encountered in the 300 fishes used for cytological work unless the females of class 3 be so considered. Careful study shows conclusively that they are not stationary but transitional forms, and that they are in stages between a completely disintegrated ovary and the origin of the male gonad—*i.e.,* the late stage tubule formation. In the second place, breeding experiments have shown that there are no sterile individuals unless diseased, which, as I have pointed out, are not very common. There are several cases where a fish has been selected as a normal female and placed in an aquarium with a male for breeding purposes and after several months two well-formed males have been found in the aquarium.

Finally the evidence that such retrogressive females may develop into males may be summarized as follows:

1. The sex-ratios described above, in which the ratio is reversed at maturity from what it was in immature stages, favors very strongly the idea of sex-inversion.

2. The occurrence of a bifurcated testis in certain males (see p. 60) which is to be explained as a connecting link between female and male.

3. The irregular contour of the testis and the large and irregular tubules of the presumed arrhenoids (Figs. 40–41) contrast conspicuously with the normal testis (Fig. 38).

4. The condition of the anal fin is further supporting evidence. Making ample allowance for all errors, the average fin ratio of the retrogressive female is far into the domain of the male (Table I.). In fact, the general appearance of such an anal fin suggests the male condition in the stage of late tubule formation, whereas there is nothing more than the remains of the epithelium of the ovarian cavity.

5. The form index and the advanced age of the arrhenoid fish as compared with normal sex-differentiation of the male are additional arguments in favor of sex-inversion in *Xiphophorus helleri.*

The opinion of breeders and fish fanciers has been for a number of years in favor of sex-inversion in *Xiphophorus helleri* and related forms. Their observations have been confined naturally to external features; mainly to form index and anal fin. I have had the opportunity to meet the members of the Chicago Aquarium Club and hear reports on first-hand observations dealing with the problem. Several members of this club have favored me with material of various kinds for which I wish to express my sincere appreciation.

Several reports dealing with sex-reversal in *Xiphophorus helleri* have appeared in *Wochenschrift für Aquarien und Terrarien Kunde.* The following may be cited from No. 17, August, 1920, p. 273: "Eine längere Abhandlung bringt Herr A. Poser über die Umbildung von Weibchen in Männchen bei Helleri. Herr P. welcher ganz der Absicht des Herr Dr. Mertens ist ("Bl." 20, No. 13) wird von anwesenden Mitglieder der Erweis erbracht, dass auch bei uns diese Umbilding bei roten H. beobachted wurde. Eine Verwechlung mit anderen Jungtieren anderer Weibchen ist ausgeschlossen, da nur ein Exemplar vorhanden war. Die Umbildung vollzog sich sehr langsam."

X. COMPARATIVE.

It may be of interest to submit a brief comparative summary of some of the publications dealing with the instability of sex in animals. This will be done under three headings: sex-inversion in teleosts, sex-inversion in other vertebrates, and the origin of definitive germ cells.

1. *Sex-inversion in Teleosts.*

As far as the writer has been able to ascertain the first report on arrhenoid fishes was furnished by Herzenstein (1891). The report concerned *Gymnocypris potanini* and *Schizopygopsis Güntheri,* both cyprinidont fishes. The observation was based on secondary sex characters—*i.e.,* on the assumption by the female of the secondary sex characters of the male.

Philippi (1904) reported "Ein neuer Fall von Arrhenoidie" in the viviporous teleost *Glaridichthys caudimaculatus,* which he describes as follows: "Ich isolirte anfangs October zwei anscheinend trächtige Weibchen von G. caudimaculatus zwecks besser Beobachtung. Während das eine an 17 October Junge warf, zeigte das andere drei oder vier Tage vor diesen Datum eine Veränderung an der Analflosse, die aber so schwach war, dass ich über ihr Wesen nicht ins Klare kommen konte. Am 17 October war diese Veränderung so weit vorgeschritten, dass sie als schwache, aber deutliche Verlängerung der vorderen Strahlen erkennbar war. Am 7 November war die Analflosse bereits bis auf etwas das Dappelte des Normalen Ausgezogen, so dass die der eines halberwachsenen Männchen in der Form glich."

In 1908 the same author reported three more cases of arrhenoidy, all of which were *Glarydichthys januarius*. In all three the form index was strictly female, while the gonopod was well advanced. After one of them died it was examined microscopically and showed total absence of a gonad, while the duct was typically oviduct. It is clear that this particular fish was in a late retrogressive stage, when it is very difficult to isolate the epithelium of the ovarian cavity. In one of the remaining two Philippi found the following peculiarities: "Makroskopisch liess dieses 3, ausserlich in bezug and die grösse ganz als Weibschen erscheinende Tier 2 nicht miteinander verschmolzene milchweisse Hoden erkennen, in deren einem 2 dottergelbe grosse Eier sich befanden und die beide einem typischem Oviduct Aufsassen."

Newman (1908) reported on a case in *Fundulus majalis*, which he at the time called "A significant case of hermaphroditism in fish." As the specimen had advanced toward maleness in morphology and behavior during the period of observation, and as *F. majalis* is decidedly bi-sexual, the case is apparently one of sex-inversion.

2. *Sex-inversion in Other Vertebrates.*

The work by Brandt (1889) on birds is of considerable interest. The name "arrhenoidie" was coined to replace "Hahnenfedrigkeit." It deals mostly with the domestic fowl, but observations on game birds, etc., are not uncommon. Birds that have laid eggs and have otherwise appeared and acted like females have been observed to assume the appearance and behavior of the male sex. Such changes occur most commonly with senility, but this is not necessary, for birds of one year of age are known to transform. It has been noted that some abnormalities, such as a solid or blind duct, occur, but there are many with no such apparent causes. Cytologically various stages of disintegration of ovarian structures and new formation of apparently testicular tissues go hand in hand. The differentiation of seminiferous tubules first begins as solid cords which luminate and may develop from one to several layers of epithelium. Such tubules show signs of spermatogenesis; no spermatozoa, however, have been encountered.

Work of somewhat similar character has been described by Pearl and Curtis (1909), Boring and Pearl (1918) on domestic fowl, and Pearl and Surface (1915) on the cow.

An interesting case of complete sex-inversion in Tritons was reported by Champy (1921). Here the inversion was from male to female, which is generally considered uncommon. The Triton in question was used for breeding purposes as a male. The transformed Triton had a typical oviduct and juvenile ovary containing numerous oöcytes which were in the process of yolk formation. Champy's conclusion is as follows: "Ensomme nous avons chez un animal adulte l'etat ovarien d'une femalle jeune. Etant Dannee l'histoire anterieure de l'animal, il n'est pas doutex que nous avons un cas d'interversion sexuelle totale."

Frogs and toads have long been known to show peculiarities as to sex conditions. Many of such abnormalities or "hermaphrodites" have been described by various observers. Lately Crew (1921) has shown that all of the "abnormalities which have been recorded can be so tabulated that the first case most nearly approximates to the normal female and the last the typical male, with respect to the nature of both primary and secondary sex characters. Thus arranged it is seen that the cases furnish an almost complete series of gradations which range from an individual almost completely female to one almost completely male, and that the conditions found readily appear to be merely graded stages of a single process."

Witschi ('21), after a thorough investigation of the sex conditions in frogs, concludes as follows: "Die Froshzwitter sind stets genetische Übergangsformen zwischen den reinen Geschlechtern (Übergangshermaphroditen), und zwar geht die Entwicklung von weiblichen zum männlichen Geschlecht."

3. *Origin of Definitive Germ Cells.*

It was pointed out in the foregoing pages that in the teleost a number of investigators have observed the origin of the definitive germ cells from peritoneal derivatives. Such conditions have been reported by Hoffmann (1886), Böhi (1904), Wallace (1904), and Philippi (1908).

Swingle (1921) reported that the lineal descendants of the primordial germ cells in *Rana catesbeiana* degenerate. As to the origin of the definitive germ cells, he says: "In the interval between the first and second larval sexual cycle following the degeneration of large numbers of maturation cells the gonad becomes filled with small cells which, because of their size, nuclear structure, and staining capacity, appear as transition stages between mesothelial cells (germinal epithelium and sex-cord elements) and true germ cells. The later history shows them to be germ cells, but their origin is open to two interpretations and is not as clear as could be desired. The writer considers these cells as small germ cells descendants of the primordial sexual elements, and not as transformed germinal epithelium elements, but admits that the evidence from his material is equally strong for the germinal epithelium viewpoint."

Clearer cut results have been obtained by Firket (1920) in the albino rat. The primordial germ cells disintegrate and have disappeared entirely in the testis of the albino rat from the tenth to the fifteenth day after birth. The origin of the definitive germ cells in the albino rats, according to Firket, are as follows: "At the time the first spermatogonia appear they are easily recognizable by the texture of their nuclei and are very numerous. This has been shown very distinctly in the same species by Hoven. Let us insist that those spermatogonia can only be derived from the small epithelial cells, as they are at this stage the only type of cells present in sufficient numbers in the sex-cord. The spermatogonia must be called 'secondary germ cells.'"

It was stated previously (p. 47) that the data presented in this paper comprise only a part of the study of the sex problem of *Xiphophorus helleri*. Particular interest is attached to the experimental work in progress at the present time. Closer cytological study of the material is imperative to elucidate difficulties encountered in cell division, chromosome composition, etc. A discussion of the bearing of the present data on the theory of sex will be postponed until the completion of the entire problem.

XI. Summary and Conclusions.

1. At birth the young fish measure on the average 8 mm. in total length and are on the verge of sex-differentiation.

2. The gonads of the indifferent stage are paired and widely

apart, suspended in a peritoneal sac immediately below the air bladder.

3. The indifferent gonad consists of two kinds of cells: primordial germ cells and cells of peritoneal origin. Both are evenly distributed in the gonad.

4. At 10 mm. in length sexes are distinct. In females the indifferent gonad develops into an ovary without any marked morphological changes of the gonad except that the primordial germ cells gradually enlarge and become oöcytes.

5. In males distinct changes take place in which the germ cells and the peritoneal cells are segregated, the former on the periphery of the gonad, the latter occupy the median and inner portions of the gonad.

6. The paired gonad in the female fuses to form a single ovary. The median surface of the fusing gonads become the ovarian cavity.

7. In the male the gonads remain paired permanently.

8. Both the oviduct and the sperm duct are formed from two sources: posteriorly from the peritoneal lining of the body cavity and anteriorly from the gonad.

9. All females from about 12.5–26 mm. in total length are subject to retrogression. To all appearances all primordial germ cells disintegrate. Definitive germ cells come from peritoneal cells.

10. Completely disintegrated ovaries have been found with epithelium of the ovarian cavity appearing from very inactive to very active. The anal fin in such cases is in early stages of transformation into a gonopod.

11. The fate of the primordial germ cells in the male is uncertain. Definitive germ cells originate from peritoneal cells.

12. Bifurcated testes occur in a large number of cases, which are supposed to originate from a completely disintegrated ovary.

13. Sex-ratios are reversed from immature to mature condition.

14. Differential viability is in favor of the female—*i.e.*, the female is at least as sturdy as the male.

15. The material decidedly favors sex-inversion in *Xiphophorus helleri*. This takes place most commonly in immature fishes, but may occur in adult animals.

16. The transformation of the anal fin into a gonopod takes

place after differentiation of the testis in normal males, but before differentiation in transformed males.

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EXPLANATION OF PLATES.

All the drawings have been made with the aid of an Abbe Camera. The lenses used were of Zeiss make and the magnifications follows the description of each figure.

Plate I.

Fig. 1. Transverse section of indifferent gonad of a specimen 9 mm. in total length. × 1000.

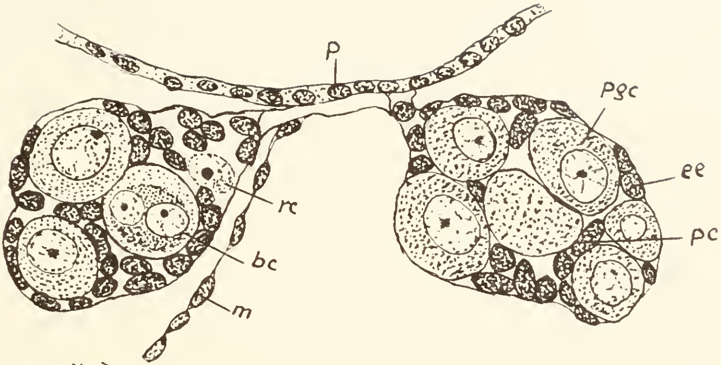
Fig. 2. Transverse section of an early normal ovary. × 1000.

Fig. 3. Transverse section of an indifferent gonad of an embryo 3.2 mm. total length. × 1000.

Symbols:

bc	Binucleated germ cell.
ec	External epithelium of gonad.
ln	Labulated nucleus of germ cell.
m	Mesentery.
mo	Mesovarium.
p	Peritoneum.
pc	Peritoneal cell.
pgc	Primordial germ cell.
rc	Red blood cell.
yg	Yolk globules.

1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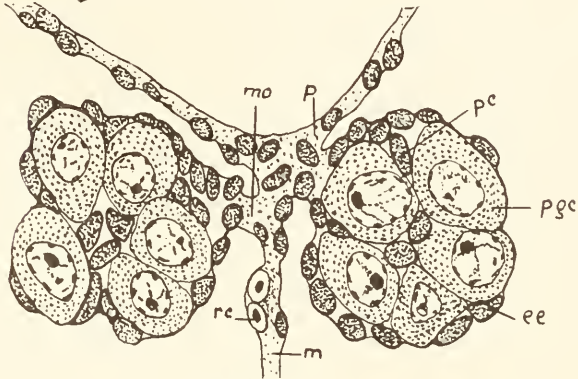
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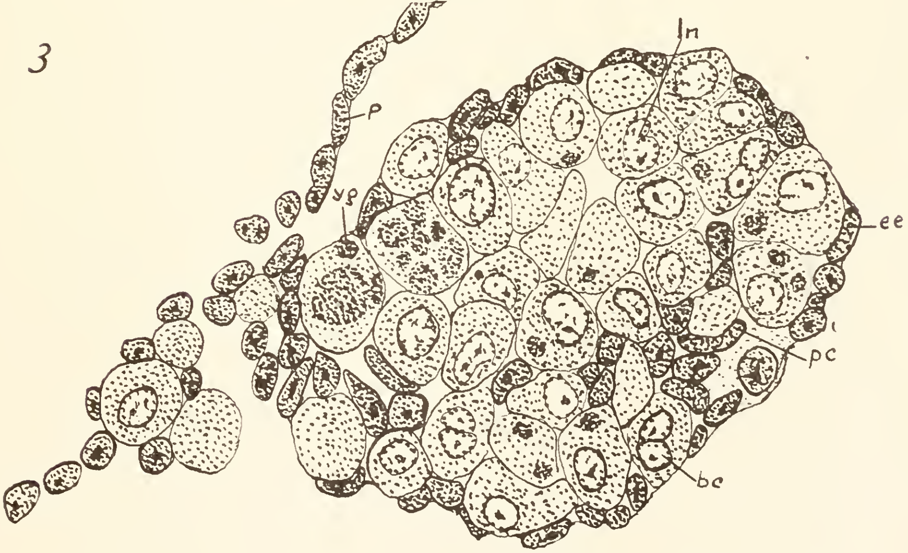
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PLATE II.

Note: All drawings of this plate are made from a preparation of a young normal ovary of a single individual.

FIGS. 4–5. Transverse section of anterior and posterior parts of ovary. × 115.

FIG. 6. Transverse section of the anterior primordium of oviduct through the anterior region. × 115.

FIG. 7. Transverse section of the anterior primordium of oviduct through middle region. × 115.

FIG. 8. Transverse section of the anterior primordium of oviduct through posterior region. × 115.

FIG. 9. Transverse section of central portion of oviduct showing a solid cord of cells. × 115.

FIG. 10. Transverse section of posterior primordium of the oviduct through the anterior region. × 115.

FIG. 11. Transverse section of posterior primordium of the oviduct through the posterior region. × 115.

Symbols:

ee	External epithelium of gonad.
epo	Epithelium of ovarian cavity.
ft	Fatty tissue.
lo	Lumen of oviduct.
m	Mesentery.
mo	Mesovarium.
nt	Nest of primordial germ cells.
o	Ovum.
oc	Ovarian cavity.
od	Oviduct.
p	Peritoneum.
pgc	Primordial germ cells.
ppo	Posteriorly extending portion of ovary.
rt	Rectum.
thm	Thickened portion of mesovarium.
ub	Urinary bladder.
ur	Ureter.
urt	Urethra.

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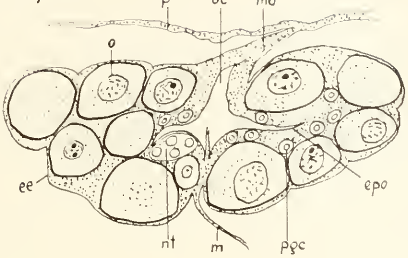
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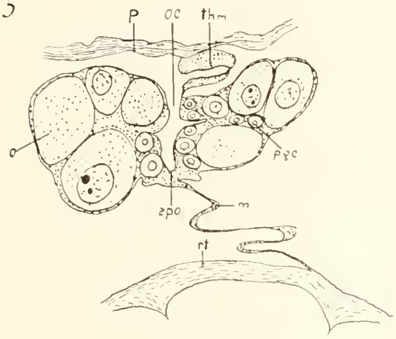
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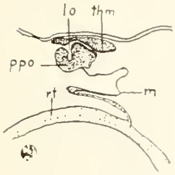
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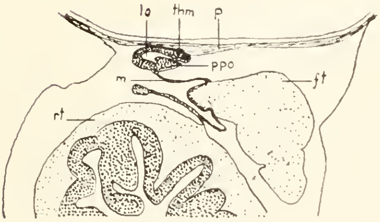
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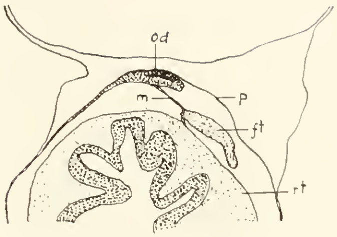

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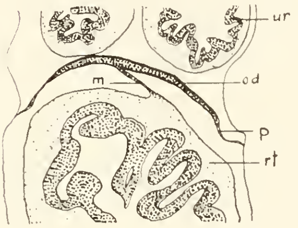

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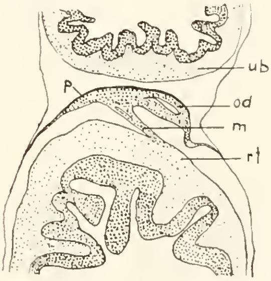

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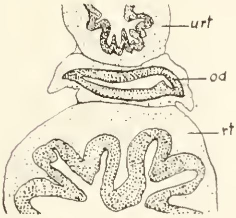

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Plate III.

Fig. 12. Portion of transverse section of epithelium of the ovarian cavity showing transformation of epithelial cells into definitive germ cells. × 1000.

Fig. 13. Section of medium-sized ovum to show migration of nucleolus from nucleus into cytoplasm. × 410.

Fig. 14. Portion of a transverse section of a young female between anterior and posterior primordia of oviduct showing no central duct formation. × 1000.

Fig. 15. Portion of a section of a disintegrating ovum showing disorganized follicular epithelium. × 1000.

Fig. 16. Section of medium-sized ovum to show migration of nucleolus towards periphery of ovum. × 416.

Symbols:

a–f	Various stages of transformation of epithelial cells into definitive germ cells.
bv	Blood vessel.
ec	Epithelial cell.
fe	Follicular epithelium.
m	Mesentery.
mno	Migrating nucleolus.
no	Nucleolus.
oc	Ovarian cavity.
p	Peritoneum.
rt	Rectum.
thf	Theca folliculi.
ub	Urinary bladder.
y	Yolk.

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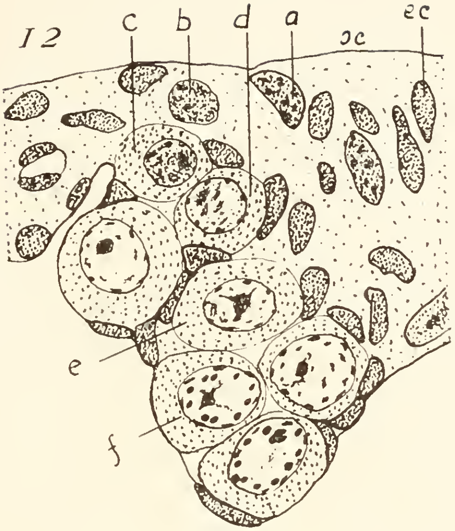
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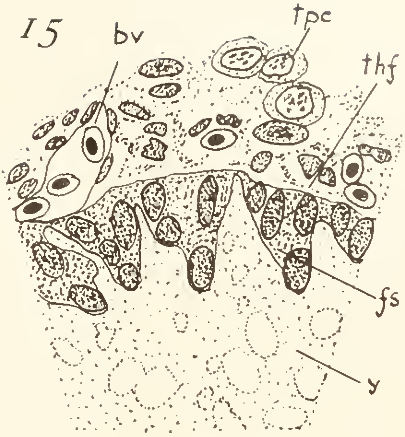
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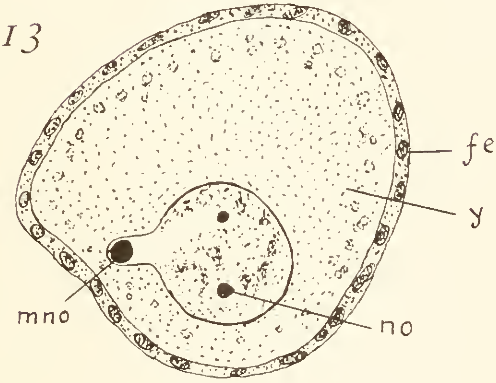

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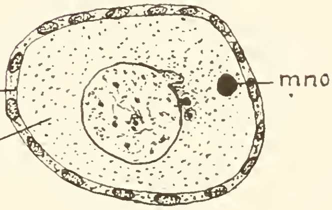

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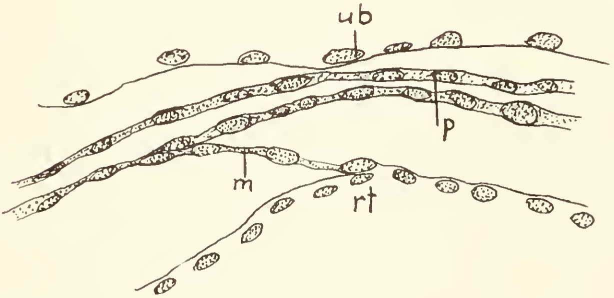

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PLATE IV.

FIGS. 17–24. Portions of transverse section of the epithelium of the ovarian cavity showing transformation of epithelial cells into definitive germ cells. × 1600.

Symbols:

a–f Various stages of transformation of epithelial cells into definitive germ cells.
oc Ovarian cavity.

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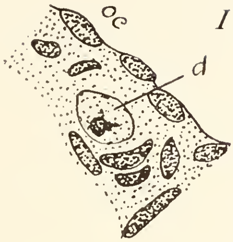

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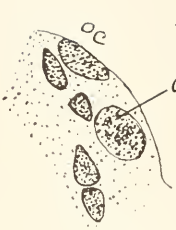

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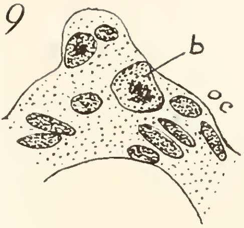

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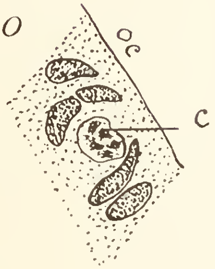

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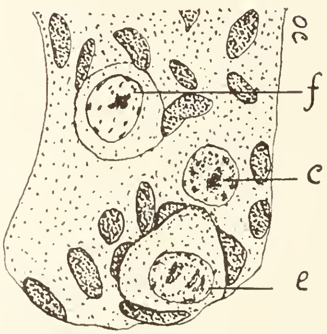

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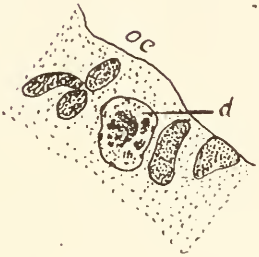

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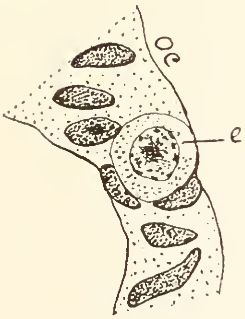

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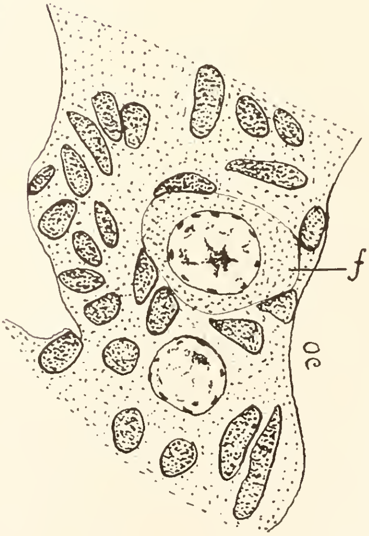

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PLATE V.

FIG. 25. Transverse section of retrogressive ovary in class 1. × 80.
FIG. 26. Transverse section of retrogressive ovary in class 2. × 140.
FIG. 27. Transverse section of normal mature ovary. × 22.5.
FIG. 28. Transverse section of retrogressive ovary in class 3. × 205.
FIG. 29. Transverse section of oviduct of ovary in Fig. 28. × 115.

Symbols:

bv	Blood vessel.
ctc	Connective tissue coat.
dfe	Disorganized follicular epithelium.
dno	Disintegrating ovum.
ec	External epithelium of gonad.
epc	Epithelial coat.
epo	Epithelium of ovarian cavity.
fed	Follicular epithelium of disintegrated ovum.
oc	Ovarian cavity.
mc	Muscular coat.
o	Ovum.
oo	Oöcyte.
ro	Remains of disintegrated ova.

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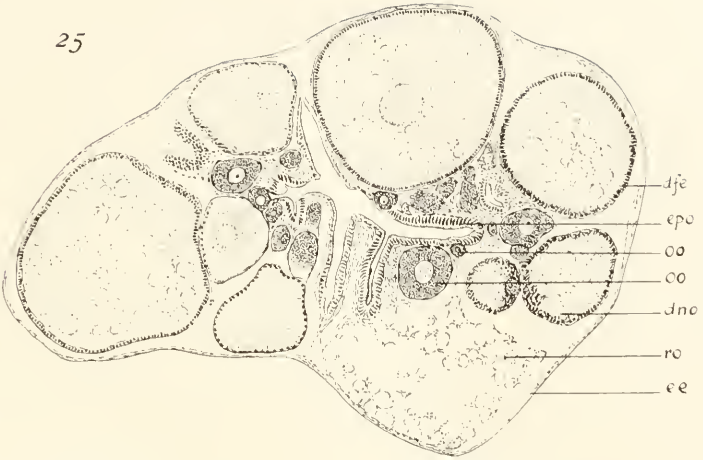
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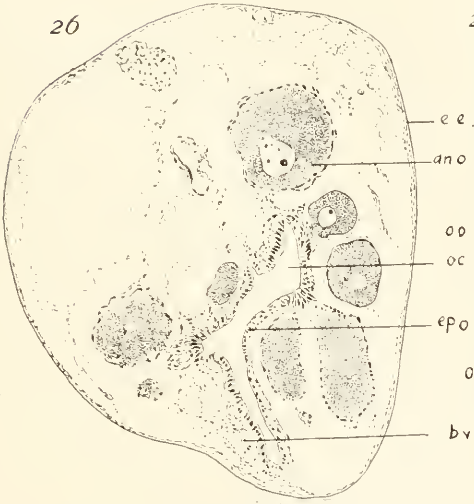
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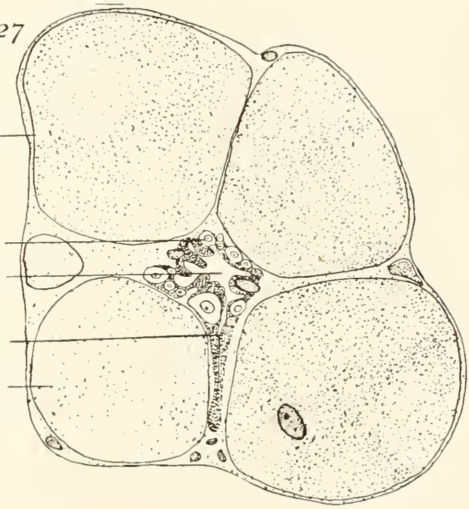

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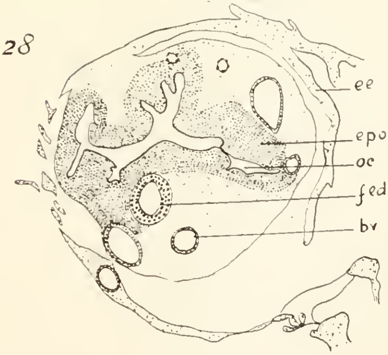
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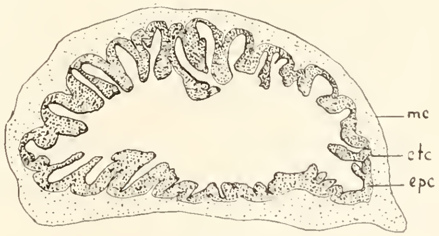

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PLATE VI.

FIG. 30. Transverse section of testis in early stage of tubule formation. × 1000.

FIG. 31. Transverse section of testis in middle stage of tubule formation. × 1000.

FIG. 32. Transverse section of testis in late stage of tubule formation (early phase). × 1000.

Symbols:

ee	External epithelium of gonad.
m	Mesentery.
mch	Mesorchium.
nt	Nest of primordial germ cells.
p	Peritoneum.
pc	Peritoneal cells.
pgc	Primordial germ cells.
rc	Red blood cell.
sc	Sex cords.

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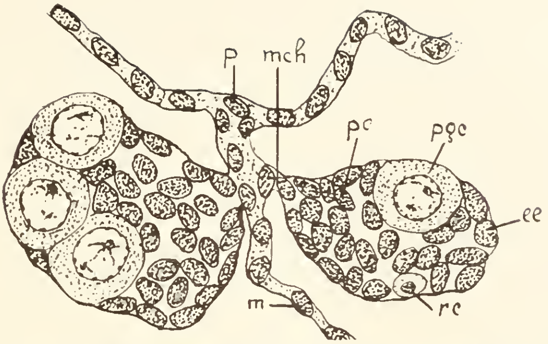
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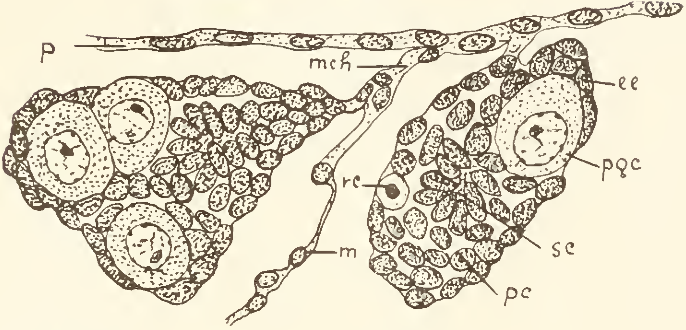
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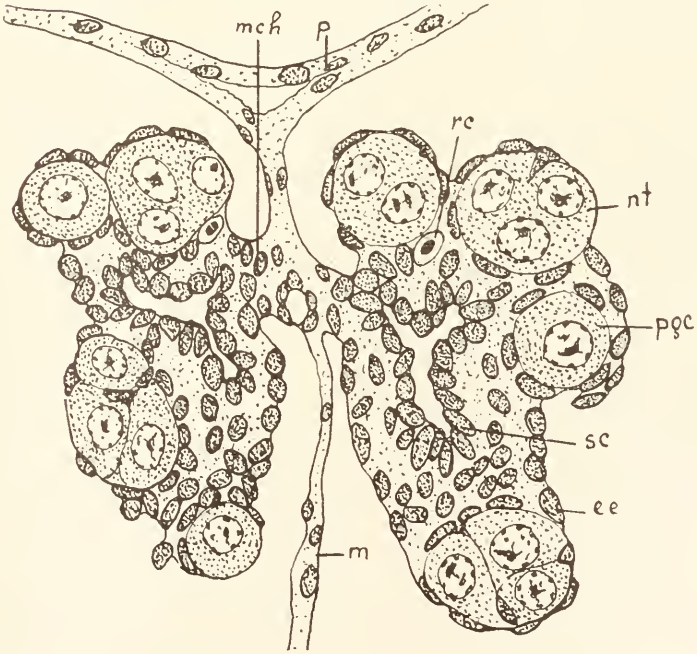
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PLATE VII.

FIGS. 33–36. Transverse section of radial tubules to show transformation of tubule epithelium into definitive germ cells. × 1600.

Symbols:

et	Epithelium of tubule.
fgc	Fully formed germ cells.
mp	Membrana propria.
lrt	Lumen of radial tubule.

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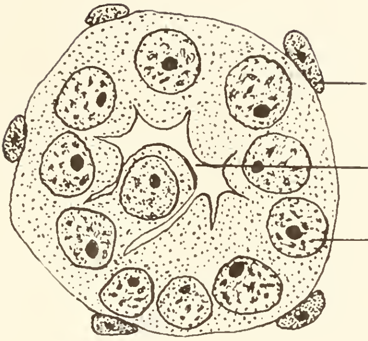

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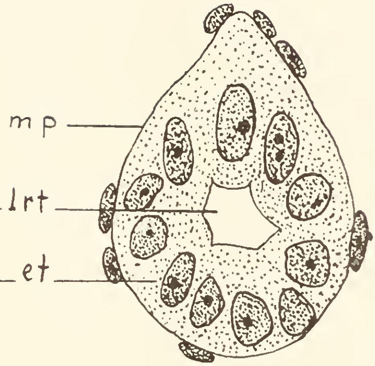

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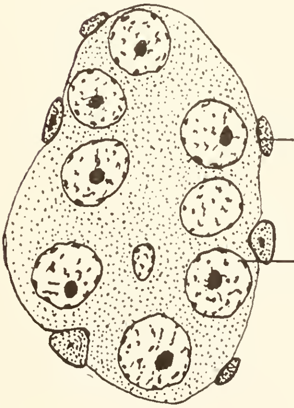

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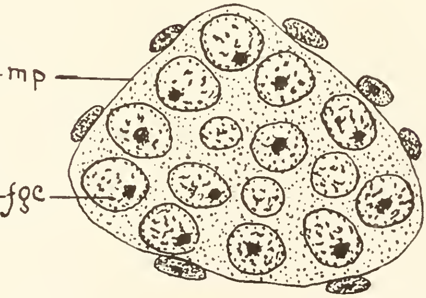

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PLATE VIII.

FIG. 37. Portion of transverse section of adult testis showing spermatogenesis. × 410.

Symbols:

ppgc	Primordial germ cells on periphery of testis.
sds	Spermatids.
sph	Spermatophore.
spz	Spermatozoa.
ssc	Secondary spermatocytes.

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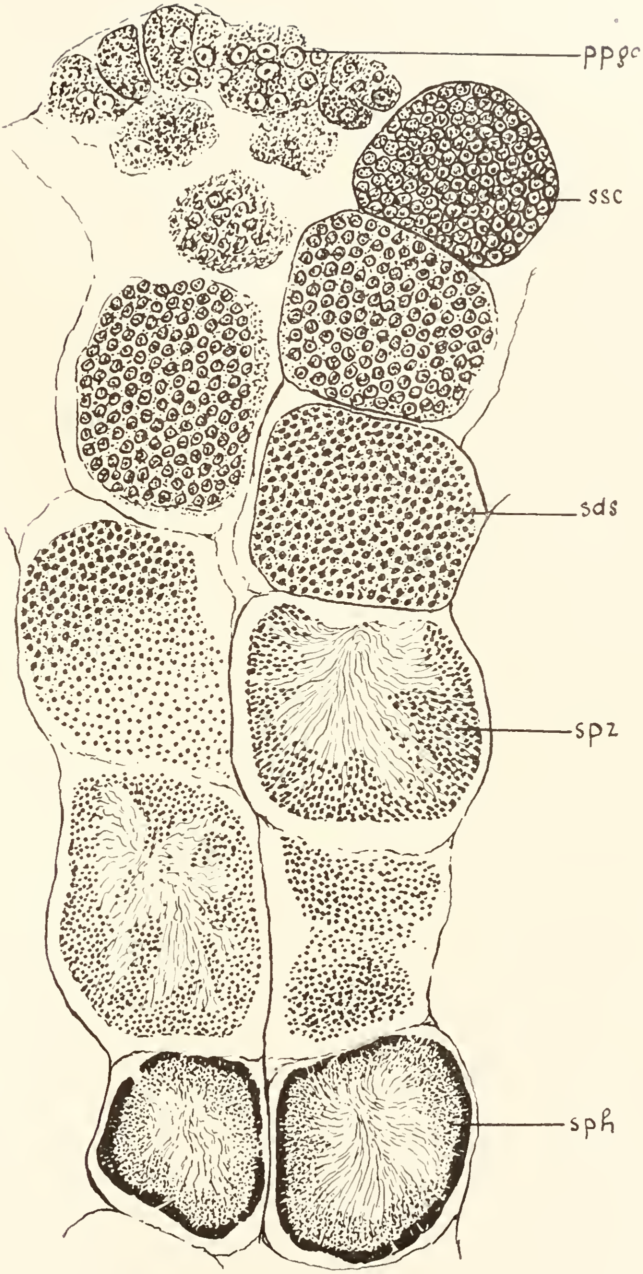
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Plate IX.

Fig. 38. Transverse section of testis in late stage of tubule formation (late phase). × 135.

Fig. 39. Portion of transverse section of testis in late stage of tubule formation to show branching tubules. × 205.

Fig. 40. Transverse section of bifurcated testis, anterior part. × 467.

Fig. 41. Transverse section of bifurcated testis, posterior part. × 467.

Fig. 42. Anal fin of indifferent stage. × 55.

Fig. 43. Anal fin of adult female. × 10.

Fig. 44. Transverse section of adult gonopod from level *A–A,* Fig. 46. × 25.

Fig. 45. Transverse section of adult gonopod from level *B–B,* Fig. 46. 25.

Symbols:

ac	Acinus or spermatocyst.
bsc	Branch of sex cord.
bv	Blood vessel.
d4r	Dorsal branch of fourth ray.
ee	External epithelium of gonad.
fep	Fin epithelium.
m	Mesentery.
mch	Mesorchium.
ntc	Nest of definitive germ cells.
p	Peritoneum.
ppgc	Primordial germ cells in periphery of testis.
sc	Sex cords.
sp	Spoon-like structures.
spd	Sperm duct.
t3r	Teeth of third ray.
t4r	Teeth of fourth ray.
v4r	Ventral branch of fourth ray.
1r–10r	First to tenth rays of anal fin.

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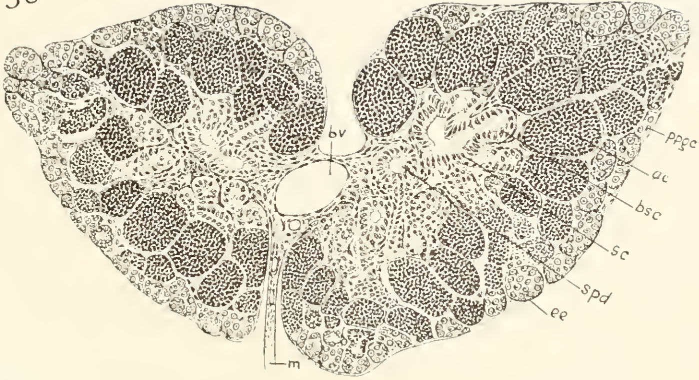
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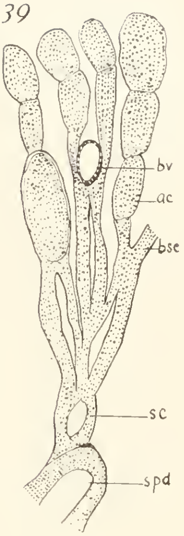
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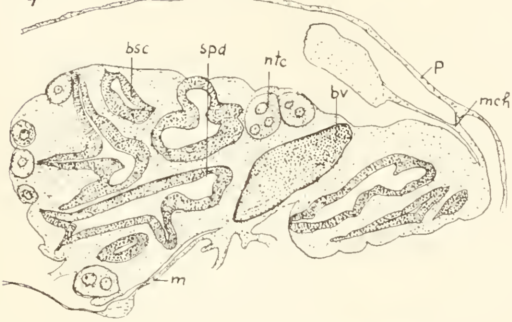
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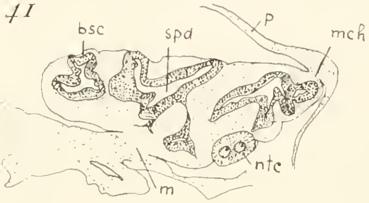
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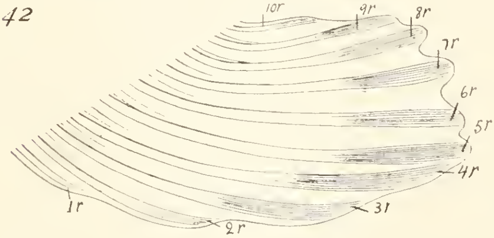
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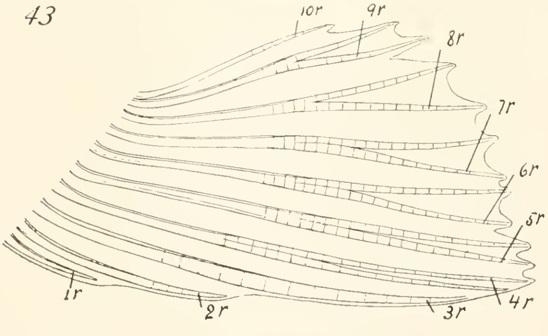
43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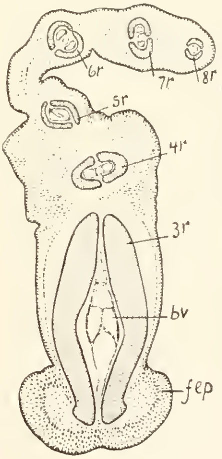
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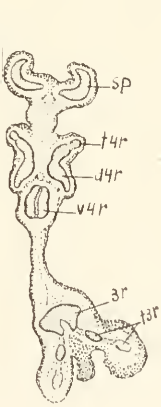
sp
t4r
d4r
v4r
3r
t3r

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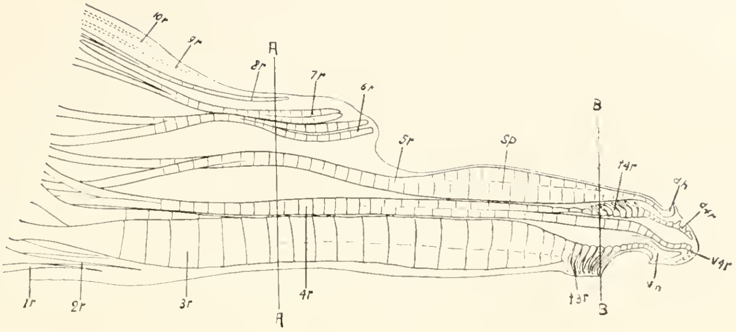
PLATE X.

FIG. 46. Adult gonopod. × 17.

FIG. 49–47. Gonopod in various stages of metamorphoses. × 17.

Symbols:

dh	Dorsal copulatory hook.
d4r	Dorsal branch of fourth ray.
kn	Knobs.
sp	Spoon-like structure.
t3r	Teeth of third ray.
t4r	Teeth of fourth ray.
vh	Ventral copulatory hook.
v4r	Ventral branch of fourth ray.
1*r*–10*r*	First to tenth rays of anal fin.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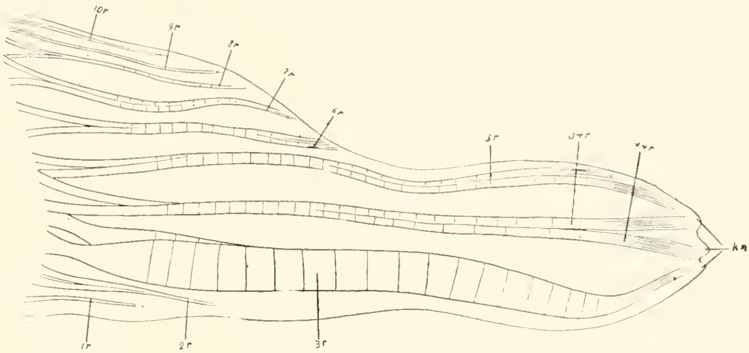
47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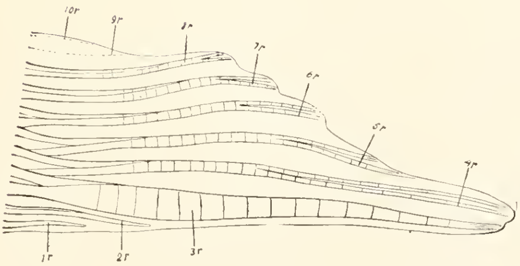
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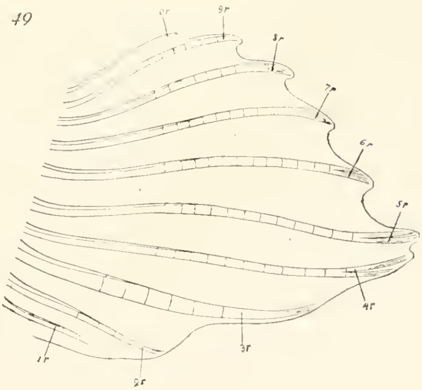
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J. M. ESSENBERG

Vol. XLV. *August, 1923.* *No. 2.*

BIOLOGICAL BULLETIN

SELECTIVE COUPLING OF GAMMARIDS.

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The result of a method of breeding involving the formation of pairs according to some system of assortment, rather than upon a purely random basis, is readily seen to be of great importance, not only for the foundation of racial diversities, but also for the conservation of genetic stability (Romanes, 1906; Pearl, 1907[a]; Wright, 1921). Actual instances, however, of assortative mating, occurring under natural circumstances, have been little studied. And the matter is of interest beyond its strictly genetic bearings; for it is probable that through sexual coupling selective with respect to somatic features, there may in different species be achieved various automatic, adaptive, consequences non-genetic in character, but nevertheless significant racially (Crozier, 1918). In this respect selective pairing of protozoans and of metazoans may differ greatly as to their implications. The selective combination of gametes (*cf.* Jones, 1920) is a question quite distinct from that of assortment of individuals, and the two should not be confused.

Among metazoans relatively few cases of normal selective mating have been recognized, although Jennings (1920, p. 193) remarks that the propensity for like to mate with like is probably in some degree quite a general phenomenon. In *Paramecium* and allied ciliates, which have been most extensively investigated, there is a well-defined tendency toward conjugation between individuals resembling one another in size and in fission-rate, and to this extent at least structurally and physiologically akin (Pearl, 1907[a]; Jenning, 1911, 1920; Watters, 1912). This is in large part due to the fact that the mutual fitting of two individuals, requisite for conjugation, is mechanically possible only when these individuals are closely similar. A like explanation holds for the selective pairing of the nudibranch *Chromodoris zebra* (Crozier, 1918, 1920); and perhaps also in the case of *Leptinotarsa,* although here Tower's (1906, pp. 238–243) account of the matter is not at all clear. Attempts to discover selective breeding involving other than size characters, among insects for example, have not been very successful. Kellogg (1906) describes observations upon 54 matings of *Hippodamia,* interpreted by him to signify that with respect to color pattern pairing is entirely at random. As Pearl (1907^b) remarks in a review of Kellogg's note, the observations, on the contrary, actually do indicate possible assortment.

For preliminary study of pairing in forms possible to breed in the laboratory, we have examined naturally occurring couples randomly taken of two species of Gammarids: *Gammarus locusta* (Linn.) and *Dikerogammarus fasciatus* (Say). Sixty-one pairs of *Gammarus* were obtained from one spot on the Staten Island shore of Raritan Bay; and seventy-one of *Dikerogammarus* were taken from the Raritan River at a point about a mile above New Brunswick.

It is possible to study size relations of members of the breeding pairs because the female is carried about by the male for a considerable time, fecundation occurring after an ecdysis by the fe-

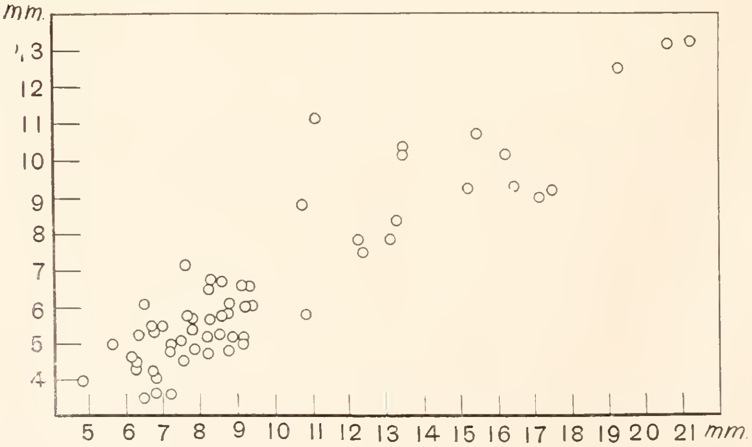

FIG. 1. Relation between the lengths of males and associated females in 62 pairs of *Gammarus locusta;* measurements in *mms.,* for each pair; ordinates, lengths of females; abscissas, lengths of males.

male. The length of each member of every pair secured was measured under low magnification, with the aid of an ocular micrometer; the total length, from anterior margin of cephalothorax to posterior margin of last abdominal segment, was measured along the curved dorsal outline.

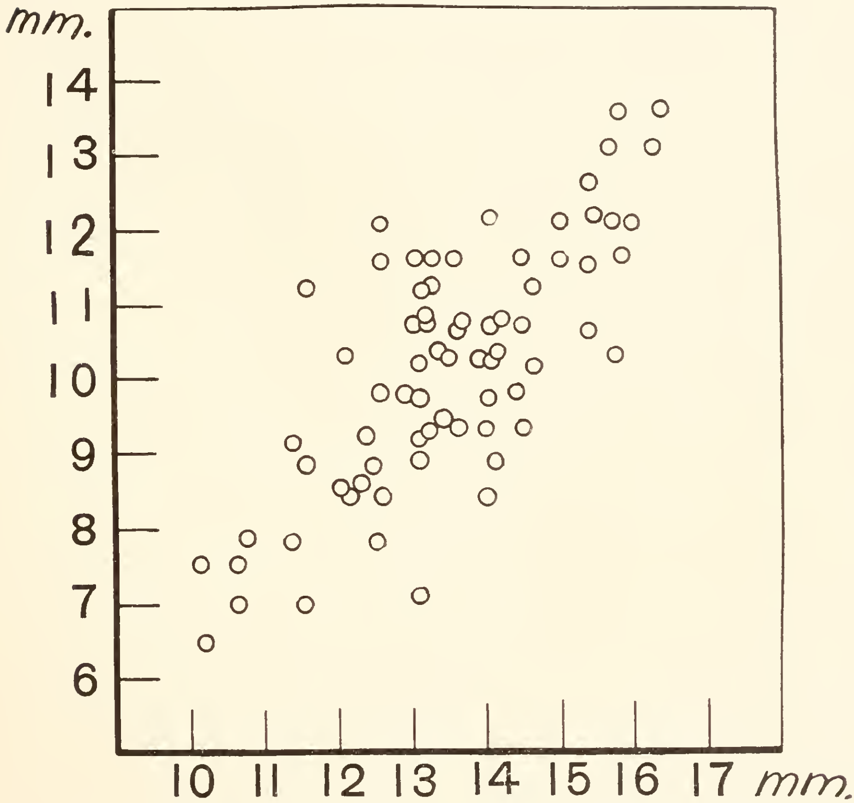

Fig. 2. Relation between length of male and of associated female in 71 pairs of *Dikerogammarus fasciatus;* lengths in *mms.*, for each pair; ordinates, lengths of females; abscissas, lengths of males.

The relation of the length of males to that of the associated females, in *Gammarus locusta,* is shown in Fig. 1 (Snyder and Crozier, 1922). Fig. 2 contains the corresponding observations for *Dikerogammarus fasciatus.* There is in each instance a high degree of selective coupling on the basis of length. For *G. locusta* the correlation index for size among members of mating pairs is

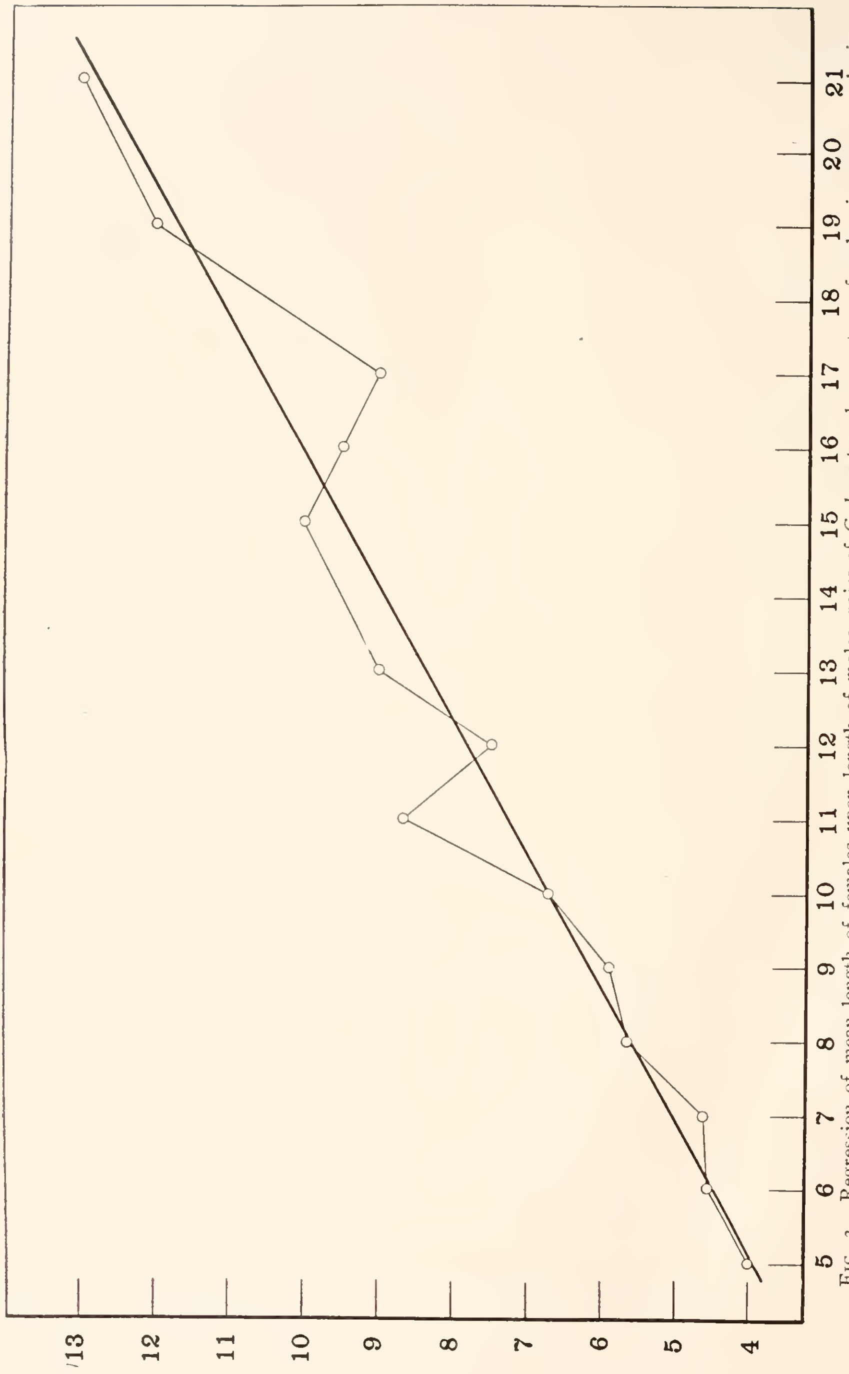

FIG. 3. Regression of mean length of females upon length of males, pairs of *G. locusta*; class centers of males, in *mms.*, as abscissas; ordinates, mean lengths of associated females.

$r = 0.914 \pm 0.014$; for *D. fasciatus*, $r = 0.690 \pm 0.042$. Figs. 3 and 4 give the fitted lines of regression for mean lengths of the females associated with males of the corresponding length classes.

The formation of breeding pairs, according to Holmes (1903) and others who have studied the question of "sex-recognition" in gammarids and among other crustaceans,[1] is brought about in a purely mechanical way. The initial encounter of male and female is by accident. Males tend to clasp objects with which they come into contact. A male seized by another male struggles until freed. A female, on the contrary, is passive when clasped, with abdomen and thoracic legs flexed, the whole body compact. This account we can confirm for the species dealt with in our measurements. There is clearly a good mechanical reason for the formation of couples in which large males carry large females, and small males carry small females. Holmes (1903) observed that a small male

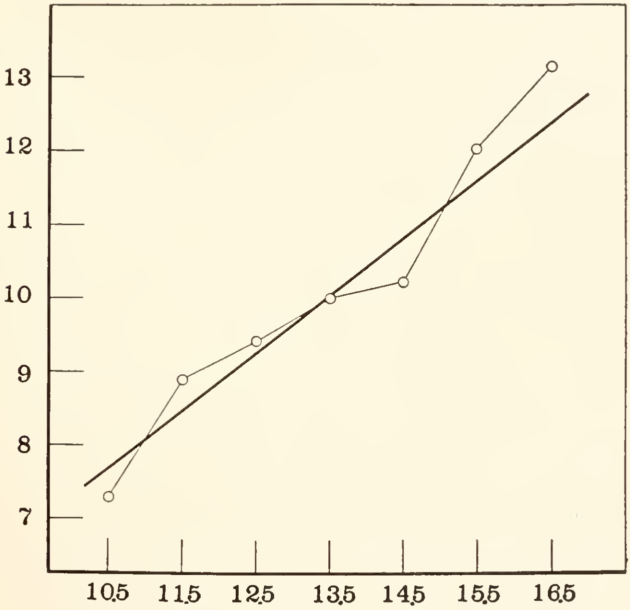

FIG. 4. Regression of mean length of females upon length of males, pairs of *D. fasciatus*, lengths in *mms.*, class centers of males, abscissas; ordinates, mean lengths of associated females.

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[1] Holmes (1903, 1909); Pearse (1909); Andrews (1910); Chidester (1911).

gammarid had difficulty in carrying a large female, and could not clasp her successfully unless his appendages were able to reach around the body of the female. It is not so clear, at first sight, why small females are not found carried by large males. But closer inspection shows that the dactyls of the male pereiopods are neatly inserted under the edges of the coxal plates of the captive female. We incline, therefore, to the view that the graded correlation between the sizes of members of pairs is determined by mechanical features of the clasping process. The magnitude of the correlation indices found in these cases is in general agreement with those of the indices reported for *Paramecium* and for *Chromodoris,* in which also there is involved a purely structural adjustment.

The possible result of the selective coupling, in relation to number and size of offspring, remains to be studied. It is known that the number of eggs carried by a female gammarid varies directly with her size, hence it is not impossible that a phenomenon like that suggested in the case of *Chromodoris* (Crozier, 1918) may be involved here also. If the economical utilization of gametes be the chief consequence of selective coupling by sizes, we deal with an adaptive mechanism not necessarily involving factorial inheritance. The possibility of the latter complication can be tested experimentally. It is not out of place to call attention to another phenomenon in which selective pairing may well play an evolutionary rôle. The imaginal size of certain parasitic insects is known to be determined by the size and the rate of development of the particular species of host insect in which their larvæ grow and pupate (Keilin, 1915; Haviland, 1922). This is due to a developmental correlation between the pupation of the larval host and of its contained larval parasites. If adult insects, of the same species, differing in size through this means, practice selective mating and are by some agency compelled to oviposit in a host of the type from which they were themselves reared (*cf.* Wheeler, 1922), a basis is clearly afforded for the foundation of divergent types. This possibility may be examined experimentally; it is profitable, in the meanwhile, to study the conditions of assortative mating in a variety of types.

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THE EFFECT OF TEMPERATURE, FOOD, AND THE AGE OF THE CULTURE ON THE ENCYSTMENT OF *DIDINIUM NASUTUM*.

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FROM THE ZOÖLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.

INTRODUCTION.

Investigations on encysted organisms clearly indicate that length of life and lethal desiccation, temperature, and chemical concentration are greatly extended by encystment. Baker (1771) maintains that the rotifer, *Tylenchus*, revived after it had remained in a dried condition on the surface of grains of wheat for 27 years. Doyere (1842) found that rotifers kept for 17 days in a desiccator, followed by 28 days under the belljar of an air-pump with a pressure of only 5 to 6 cm. of mercury, and thereafter thoroughly dried in direct sunlight, and then subjected to a temperature of 140° C. were still viable. Mast says (1917): "Cysts of *Didinium* kept sealed air-tight in a 10 c.c. vial for nearly five years were still viable." Bodine found that the cysts of *Colpoda* withstand extraordinary concentrations of various acids and narcotics as well as remarkably high temperatures.

The results obtained in observations on the cause of encystment are, however, much less conclusive. Root maintains that the encystment of suctoria is due to lack of food. He says (1914): When left without food for several weeks, Podophryæ become smaller and finally encyst in situ." Mast obtained similar results in experiments on *Didinium,* but not invariably. He says (1917): "Encystment in *Didinia* can usually be induced at any time by cutting off the food supply. But it frequently occurs when there is an abundance of food present and sometimes it does not occur when there is none. This is especially true for the cultures in which conjugation has been prevented for a considerable number of generations." Miss Carter (1919) seems to think that abundant food is essential for encystment of *Amœbæ,* but that low temperature is not, although she found encysted specimens during the winter months. Miss Hogue contends that the accumulation of waste products is the primary cause of encystment. She says, referring to *Ameba limax* (1914): "The condition under which they encyst seems to point to a weakened vitality. It seems as though the digestive fluid has not been formed in sufficient quantity, owing to the rapid division. So it is not the lack of food, but rather the loss of power of assimilation." And (1917, p. 571): "When the accumulation of waste product is very great, and the *Amœbæ* have multiplied so fast that there is no further place for them to go, they encyst. I have frequently observed a culture dish containing thousands of *Amœbæ,* with plenty of food, soon become covered with cysts, the *Amœbæ* having often encysted over night."

It is generally held that encystment is a protective adaptation. Thus Calkins says (1910, p. 40): "This is a special adaptive process by which the organisms are enabled to survive when the environment is unsuitable." (P. 90) "There is a general agreement that its object is to protect the individual during periods of drought, cold, or periods of reproduction." (P. 192) "It occurs when the animal is in danger of drying, or in some cases before division, in others for the purpose of digesting a full meal." Minchin (1917) expresses similar views, as does also Jordan. Jordan says (1918, p. 73): "It serves to tide the species over a period of dryness, famine, or unsuitable temperature, and to preserve alive in a hostile environment a sufficient number of individuals until such time as favorable conditions recur. The spore-stage is in fact physiologically analogous to the periods of hibernation and estivation among the higher forms of life. In this resting state the living matter of the spore may remain dormant for years or even for decades."

It seems evident, then, that many investigators hold that encystment is induced by adverse changes in the environment, and that it protects the organism against unfavorable conditions. The evidence presented in favor of the first of these contentions is, however, not strong. A thorough experimental investigation of the relation between the environment and encystment is, therefore, highly desirable.

In this paper we shall present the results obtained in experiments on the effect of temperature, food, and the age of the food-culture on the encystment of *Didinium nasutum*.

Materials and Methods.

The didinia used in these experiments were all derived from cultures started from a stem-culture kept continuously in the Laboratory. Battery-jars were used as containers for these cultures. From time to time, as the supply of food in the culture-jars became depleted, a portion of the liquid was replaced by a corresponding quantity of a fresh, vigorous, paramecium culture. In this way the didinia were kept continuously in a flourishing and active condition.

The effect of temperature and food on encystment was ascertained as follows: Into each of five square watch-glasses, previously sterilized, was placed 3 c.c. of culture fluid containing numerous paramecia, and into each of five similar watch-glasses 3 c.c. of culture fluid taken from the same jar, but filtered so as to remove all paramecia and other organisms that might serve as food for *Didinium*. To each of the watch-glasses thus prepared there were added vigorous didinia, usually five, all taken from the same jar. Two of the watch-glasses, one with and one without food, were then placed into each of five thermostats maintained at different temperatures as indicated in Table I. All of these cultures were examined for cysts once every day until they died out. In this experiment there were consequently under observation simultaneously ten didinia cultures which were precisely the same with the exception of food and temperature. This experiment was repeated a number of times as indicated in the accompanying tables.

In a number of additional experiments there were fewer cultures and temperatures under simultaneous observation. In an extended series of experiments made after the main part of the work was completed there were only two cultures, one with and one without food, both at 27° in each experiment. Cultures containing fluid taken from the jar which had contained the didinia used in the tests were added to some of the thermostats in a few experiments. This, however, had no appreciable effect on encystment.

The results obtained in all of these experiments are summarized in Table I. These results show conclusively that at 25°–30° en-

TABLE I.

THE EFFECT OF TEMPERATURE AND FOOD ON ENCYSTMENT IN DIDINIUM.

Note that the percentage of encystment is greater in the cultures with food than in those without and that it is greatest at 25–30°, the optimum temperature for reproduction.

Temp.	With Food.			Without Food.			Ratio of Percentages of Encystment.
	No. of Cultures.	No. of Cultures Encysted.	Percentage of Cultures Encysted.	No. of Cultures.	No. of Cultures Encysted.	Percentage of Cultures Encysted.	
4–16°....	14	0	0	11	0	0	
20–23°....	26	14	53.8	22	4	18.18	3.23
25–30°....	18	15	83.3	8	3	37.5	2.22
27°.......	51	42	82.3	57	22	38.6	2.13
30–35°....	25	17	68.0	26	7	26.92	2.52
39°.......	16	6	37.5	9	1	11.1	3.38

cystment occurred in a much greater proportion of the cultures than at any other temperature, both in those with and in those without food, that at all of the temperatures excepting the lowest it occurred in a much greater proportion of the cultures which contained food than in those which did not, and that the difference in the extent of encystment in the cultures with and without food was least at 25°–30°. They show that at 39°, which is only a few degrees below the maximum, there was but little encystment, and that at 4°–16° there was none at all.

In the preceding experiments the didinia died out in relatively more cultures at the higher and the lower temperatures than at the others. At 4°–16° and at 39° they died out in over half of the cultures in two days, while at 27° they died out in less than one tenth of the cultures in the same time. Moreover, the death rate was greater at all of the temperatures in the cultures without food than in those with food. In two days at 39° two thirds of the cultures without food died out and only one half of those with food, at 30°–35° a little over one half of those without food and less than one eighth of those with food, at 27° nearly one sixth of those without food and less than one twenty-fifth of those with food, etc. Furthermore, reproduction took place more rapidly at 25°–30° than at the other temperatures. At 25°–30° the fission rate ran up in some instances to 8 a day. At 39° it never exceeded one or two a day, and at the lower temperatures of 4°–16° there was no reproduction at all.

In all of these experiments the didinia were rather suddenly subjected to the different temperatures. It was thought that the lack of encystment at the extreme temperatures might have been due to the sudden change. An extended series of tests was consequently made in which the temperature of some of the cultures was very gradually reduced to 4°–16°, and that of others very gradually raised to 39°. The results obtained in all of these tests were, however, essentially the same as those obtained in the earlier experiments.

All of the results obtained consequently indicate that encystment in *Didinium* takes place most readily under conditions of temperature and food which appear to be optimum for reproduction. The results are, however, not conclusive in reference to the question of the effect of food. An abundance of food was added in all of the experiments when they were set up and in some more was added later. In some cultures there was still an abundance of food present when encystment occurred, but in others there was none. The number of each was unfortunately not recorded. It is consequently evident that in some of the cultures which contained food encystment may have been due to absence of food. In the cultures with food there was a much greater increase in the number of didinia than in those without food. Rapid and extensive reproduction of didinia confined to a small space seems to favor encystment, and it may be that this is owing to accumulation of waste products.

In attempting to ascertain the effect of the age of the food-culture on encystment experiments were carried out as follows: Timothy hay was added to tap-water, spring-water, and distilled water in three large flasks, one gram to 100 c.c. These flasks were then kept for 30 minutes at the boiling point. After they had cooled to room temperature, usually the following day, a large number of paramecia in a small amount of liquid was added to each flask and the contents poured into battery-jars. These jars were then placed side by side and kept at room temperature. Three more cultures were prepared precisely the same way two days later and also four days later; so that at this time there were at hand three sets of paramecia cultures, one just completed, one two days old, and one four days old. A given amount of solution containing many paramecia was now taken from each of the nine cultures and put into nine square watch-glasses, each containing five vigorous didinia, all taken from the same jar. These watch-glasses were put into a thermostat kept at 27°–28°. The number of cultures in which cysts occurred was recorded daily, as well as the number of cysts in each and the condition of the paramecia.

Other sets of watch-glass cultures were prepared and treated precisely like this on the following days. A summary of the results obtained in all of these are presented in Table II.

TABLE II.

THE RELATION BETWEEN THE AGE OF THE FOOD-CULTURE AND ENCYSTMENT IN DIDINIUM.

Age of Food Culture in Days.	No. of Didinia Cultures.	No. of Cultures Encysted.	Average Time Required for Encystment in Days.	Percentage of Cultures Encysted.
0	11	2	3	18.1
1	8	5	3-2/5	62.5
2	11	4	3	36.3
3	14	7	3	50
4	14	10	3-1/5	71.4
5	20	15	3-1/5	75
6	15	14	3-1/14	93.3
7	21	11	2-6/11	52.3
8	18	12	3-1/3	66.6
9	13	9	2-2/9	69.2
10	15	7	3	46.6
11	10	4	3	40
12	10	4	2-3/4	40

By referring to this table it will be seen that as the culture medium increased in age the percentage of the number of cultures in which encystment occurred increased to a maximum, after which it decreased. It is well known that the chemical composition of protozoa cultures changes with age. It is consequently evident that the increase and decrease in the percentage of encystment noted must have been due either to this change or to a change in the quantity or the quality of the food. The amount of food was, however, practically the same in all of the didinia cultures, but the quality may have been different. It is therefore impossible to say whether the change in the percentage of encystment was due to a change in the chemical composition of the culture medium or to a change in the quality of the food. However this may be, encystment occurs freely in the culture media which are very favorable for growth and reproduction of paramecia. The maximum was found in cultures six days old, when the paramecia were very abundant and vigorous. Whether or not fission rate was at a maximum at this time was, however, not ascertained.

SUMMARY.

1. Didinia encyst most readily at a temperature of 25°–30°, which is also the optimum temperature for growth and fission.

2. They do not encyst in temperature so low or so high that it is injurious. They do not encyst at all below 16° and rarely above 39°.

3. They encyst more freely in cultures supplied with food than in those without food, but this is probably due to greater increase in numbers, resulting in greater accumulation of waste material in the one than in the other.

4. They encyst most readily in culture media, which are probably most favorable for growth and reproduction of the paramecia on which they feed.

5. Encystment serves as protection against unfavorable conditions in reference to food and temperature, but such conditions do not facilitate encystment.

6. Encystment is probably induced by the accumulation of excretory waste material.

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FEEDING REACTIONS IN THE CILIATE, *DILEPTUS GIGAS,* WITH SPECIAL REFERENCE TO THE FUNCTION OF TRICHOCYSTS.

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1. INTRODUCTION.

Food-getting is the first necessity of the living thing, and the chief end toward which the fundamental structures of the body are directed, and this, whether in the highest mammal or lowest protozoan, becomes the chief economic problem to be solved. "Food-getting, therefore, more than any other function of the body, has been the most influential in leading to morphological development." Thus wrote one of the leading investigators in the field of protozoölogy (Calkins, '10).

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[1] The work here presented was largely done at the Zoölogical Laboratory of the Johns Hopkins University, Baltimore, Md., but it has been amplified by work done at the Zoölogical Laboratory of Washington University, St. Louis, Mo., and at the Marine Biological Laboratory, Woods Hole, Mass. To the directors of these laboratories I am very grateful for facilities offered.

The feeding reactions of animals have long been a favorite study for students of animal behavior. More recently the reactions of certain protozoa have been intensively studied with the hope that such problems as the choice of food would be reduced to their simplest terms in these unicellular animals which in other respects apparently stand near the bottom of the scale of development.

Ehrenberg ('38) was undoubtedly one of the first to make observations on the ability of protozoa to choose their food. He records experiments on the ingestion of carmine by various organisms in an attempt to show that protozoa do not select their food. Entz ('88) and others of his time were, however, strong in their contention that infusoria are able to select their food, ingesting certain kinds and rejecting others in a systematic way. Bütschli ('89) supports Ehrenberg's view. He concludes that protozoa do not possess the power of choice, and Verworn ('89) likewise concludes, on the basis of rather extensive experiments, that there is no selection.

Jennings ('02) states that *Vorticella* and *Stentor* probably do not have the power of selecting their food in any precise way. Mast ('09) in his work on *Didinium* shows that the apparent choice of food on the part of this organism is due to the fact that the seizing organ will adhere to the surface of some organisms and not to others. The didinia come in contact with all sorts of objects in their random swimming and "select" as food only those to which the seizing organ will adhere.

Schaeffer ('10) in his work on *Stentor cœruleus* concludes that this organism exercises a very definite selective power and discriminates very accurately between organisms and indigestible particles, and that it discriminates even between different organisms. He contends that it selects its food on a tactual basis, and apparently not on a chemical one. The same worker ('16) reports some experiments on *Amœba* and maintains (p. 562) that although *Amœba* eats insoluble substances, there is a slow process of learning in favor of selection. He ascribes to the endoplasm of *Amœba* a more specific power of discrimination than to the ectoplasm, and also maintains that movement of an object is a very important factor in determining whether or not it shall be eaten.

Calkins ('10) says, "while most of the protozoa wait until the prey comes to them, and take what they can get, others are predatory and go in search of food. These are the most interesting of all protozoa, for they are occasionally too fastidious apparently to take the ordinary run of microscopic wilds, but seem to select their food with all the care of a gourmand." As an example of this type he describes the reactions of *Actinobolus radians.*

Miss Moody ('12) in her study of *Actinobolus* and *Spathidium* asserts that they "subsist exclusively on a special type of ciliate. *Actinobolus* awaits the coming of *Halteria grandinella* before making use of its weapons of offense," while *Spathidium* swims about "with seeming indifference to all food material except the little ciliate, *Colpidium colpoda.*" She concludes that "the protoplasm of these organisms has become modified chemically and physiologically to such an extent that a reaction to one kind of protoplasm only is possible; in other words, forms like *Actinobolus* and *Spathidium* have become "educated through 'error' to the selection of one species of food each, namely, *Halteria grandinella* and *Colpidium colpoda.*"

Metalnikow ('12) contends that if paramecia are fed for some time on a non-digestible substance, they take in gradually less and less, until finally they refuse it entirely under all conditions, but that they nevertheless take in other substances just as before. He shows that in the case of feeding on carmine this power of selection is lost at the time of division. He also shows that there is a decided power of discrimination between substances already within the body; for some substances are quickly excreted, while others remain within the body for a considerable length of time.

In his studies of one of the Suctoria, *Podophrya collini,* Root ('14) maintains that there are several definite factors which determine the selection of food in this organism. He shows that the character of the outer surface of certain organisms as to physical and chemical constitution, mucus secretion, etc., prevents the attachment of the seizing apparatus. He shows, moreover, that the size, the activity, and the characteristic behavior of certain organisms in relation to the sessile habits of *Podophrya collini* are also determining factors.

From this brief review of some of the more general literature in this field it is evident that selection of food has thus far been positively demonstrated in only a very few forms, while in general it would appear that most workers have supported the opposing view.

There are only a few incidental references in the literature to the feeding habits of *Dileptus gigas*. Bütschli ('89) says its food is "sehr grob," and is quoted by Calkins as saying that it feeds on ciliates alone. According to Wrzesniowski ('70), "*Dileptus gigas* is a voracious animal which feeds only on living food, preying especially on *Stylonychia*." Pritchard ('61) says it feeds largely on green monads, because of which it is often of a green color. Hausman ('17) says "*Dileptus* is surely the king of beasts among the ciliated protozoa. It is entirely carnivorous and its appetite is apparently insatiable. The prey is stung by well-developed trichocysts, and if too large to be swept into the buccal cavity by the cilia, it is forced in by the writhings of the neck" (proboscis).

It is clearly evident that there are a number of different views concerning the feeding habits of this infusorian, all of which are apparently based on purely incidental observations. Does *Dileptus* feed on ciliates alone, or even on living food only, which would involve the power of choice of food? Does it paralyze its prey by means of trichocysts? What is the nature of these structures? These are the problems which are considered in the observations and experiments which comprise the material presented in this paper.

The work was begun at the suggestion of Professor S. O. Mast, to whom I am deeply indebted for many helpful suggestions concerning the experiments made and for much valuable criticism during the preparation of this paper.

2. MATERIAL AND METHODS.

Dileptus gigas is one of the holotrichous ciliates belonging to the family Tracheliidæ. It is one of the largest of the more common protozoa, often measuring over 600 micra in length. It possesses an elongated body, sharply pointed at the posterior end, and at the anterior end drawn out into a long proboscis which is frequently as long as the body itself. The mouth opening is located at the basal end of this proboscis, and has a circular aperture with a short funnel-shaped gullet leading from it (Fig. 1). Both these structures are capable of enormous expansion at the time of feeding. Normally, however, they are closed except for a pit-like cavity which is always present. A cytopyge is sometimes discernible near the posterior end, at which place fæcal material often collects in a large vacuole from which it is sporadically discharged. There are numerous contractile vacuoles of which the larger ones are arranged in a series near the aboral surface. Thin contractile fibrillæ extend around the body in the form of a flat spiral, effecting about one complete turn for the entire length of the body. The entire body is covered with short cilia which run in rows parallel to the fibrillæ. On the ventral surface of the proboscis, which is somewhat flattened, the cilia are considerably thicker and longer than elsewhere, especially along the edges, where they are in the form of two bands. These extend backward in such a way as to meet and form an arch just behind the mouth opening. Since all these cilia beat backward under normal conditions, a decided current is produced in the groove between these two bands of large cilia. This current starts at the tip of the proboscis and runs back to the mouth, where it ends in a sort of vortex, due to the action of the band of cilia which partially surrounds this pit-like orifice as above described. Structures which have been quite generally described as trichocysts are found in the oral surface of the proboscis. They can be seen only indistinctly in living mate-

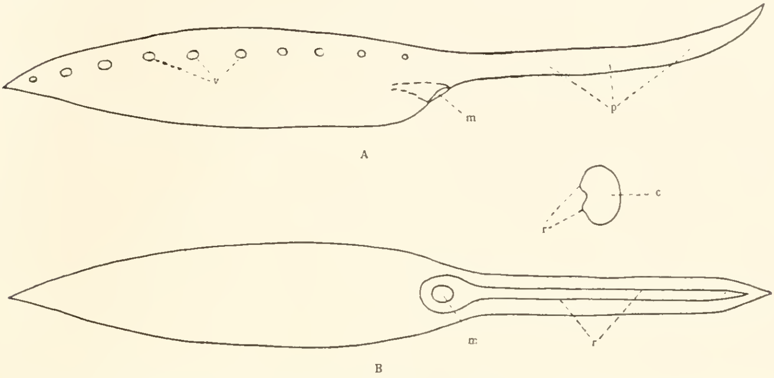

Fig. 1. Diagrammatic sketches of *Dileptus gigas:* *A*, side view; *B*, oral view; *v*, contractile vacuoles; *p*, proboscis; *m*, mouth; *c*, cross section of proboscis; *r*, bands of large cilia.

rial. There are at least several hundred of these structures, all of which are arranged in a band extending from the mouth to the tip of the proboscis, approximately in the mid-line of its oral surface.

There has been much discussion concerning the nuclear condition of this organism, but recent investigators appear to be agreed that *Dileptus* possesses a distributed nucleus. An account of this condition will be omitted here, as it is planned to present a discussion of the nuclear phenomena during the life history of this organism in a separate paper.

Dileptus is never at rest. It is always swimming, as a rule quite slowly unless disturbed. In cultures it is observed to spend most of its time swimming slowly just above the debris at the bottom of the dishes. Apparently a like condition obtains in nature, for if *Dileptus* is found in a pool, samples taken from near the bottom contain many more specimens than those taken at higher levels. It is observed to progress with its posterior end close to or in contact with the bottom, while its anterior end is always at a noticeable elevation. It proceeds with its proboscis ahead, continually waving it now this way, now that, as if in search of food. It also rotates slowly on its longitudinal axis. This rotation, in connection with the searching movements of the proboscis, enables it to explore a very large area.

In January, 1919, a few specimens of *Dileptus gigas* appeared in an old "paramecium-culture" in which active fermentation had long since ceased. It can not live long in any culture in which active fermentation is taking place, nor can it thrive in cultures rich in organic food supply. Consequently, very dilute infusions were made, and these were usually inoculated with *Euglena gracilis* before introducing *Dileptus*. In this manner the original culture was kept for more than fifteen months.

In nature I have found them in the large pond on the Homewood Campus of the Johns Hopkins University, Baltimore, and also in the "school-house" pond near the Biological Laboratory at Cold Spring Harbor. L. I. They were found also in the Cedar Swamp pond at Woods Hole and in the outlet channel of Creve Cœur Lake near St. Louis, Mo. In all these ponds at the time *Dileptus* was found there was but little organic decay taking place and the water was "relatively pure."

In preparation for the experiments described in the following pages, a number of the organisms, usually about fifteen, were transferred with a capillary pipette from the stock-culture to small dishes containing about 5 c.c. of spring water. They were left in this water without food for from 2 to 3 days. During this starvation process about ten per cent. usually encysted and the rest diminished considerably in size, became very hungry, and were consequently in excellent condition for observation on the selection of food and the process of feeding. When kept longer, without food, they continued to diminish in size until in the course of a week they were reduced to less than one tenth of their former length and probably to less than one one-hundredth of their former volume, after this they disintegrated unless this was prevented by opportune feeding.

All the experiments on the selection of food were conducted as follows: A designated number of starved dilepti, usually fifteen, were placed in a small watch-glass with about 3 c.c. of spring water. A drop of a concentrated suspension of the substance to be tested was then added and the whole thoroughly mixed. The feeding reactions of some few of the specimens were carefully observed for longer or shorter periods of time. At the end of twenty minutes all the dilepti were taken out of this medium by means of a small pipette and introduced into a like volume of spring water. They were then observed, either individually or two at a time, on a slide under a magnification of about 300 diameters, and the number of vacuoles in each was carefully counted. In this way various living organisms and numerous inanimate particles were tested.

The protoplasm of *Dileptus* is quite opaque under ordinary conditions of culture. Occasionally a culture was obtained in which the organisms were relatively transparent and it was only with such material that the feeding experiments were made, for even under optimum conditions there was some difficulty in seeing precisely what passed into the body unless it was specifically colored as, *e.g.*, carmine or india ink. Another factor which favored observation on the amount of food ingested was the characteristic large size of the first vacuoles formed, whenever an organism was introduced into a new medium, especially after a period of starvation. Such large vacuoles are very specific.

3. EXPERIMENTS ON SELECTION OF FOOD.

A. Inanimate Substances.

In experiments on inanimate substances it was desired to use only insoluble and non-toxic materials. The following substances were tested: carmine, chalk, sand, powdered glass, and india ink. Three of these, namely, carmine, glass, and india ink, appeared to be more favorable than the others, because of the fact that vacuoles filled with these substances were readily distinguishable. The results obtained in ten experiments with each of these three substances are given in Tables I., II., and III.

Table I. contains the results obtained with carmine. This table shows that only three of the one hundred and forty-nine individuals tested did not ingest carmine, that more than eighty per cent. of the total number tested formed one vacuole, while fifteen per cent. formed two vacuoles each. Thus it is evident that carmine is eaten, but only in small quantities, for in only seventeen

Table I.

Experiments on Carmine.

Table showing results obtained in feeding *Dileptus* on carmine. In the five columns under the headings 0–4, are indicated the number of individuals which formed 0, 1, 2, 3, 4, or more vacuoles containing carmine, respectively, during the twenty minutes of the experiment. Carmine was ingested by 97.5 per cent. of the individuals but in only 17 per cent. was there more than one vacuole containing carmine formed.

Experiment Number.	Total Number of Dilepti.	Number of Carmine Vacuoles.				
		0	1	2	3	4 or More.
1	15	0	14	1	0	0
2	15	0	12	2	1	0
3	15	0	8	7	0	0
4	14	0	11	3	0	0
5	15	1	13	1	0	0
6	15	1	12	2	0	0
7	13	0	12	1	0	0
8	15	1	11	2	1	0
9	16	0	13	3	0	0
10	16	0	14	1	1	0
Average	14.9	.3	12	2.3	.3	0
Per cent.		2	50.5	15	2	–

per cent. of the individuals tested was there more than one vacuole containing carmine formed.

Table II. contains the results of ten experiments with powdered blue glass. It shows that eighty-nine per cent. were observed with vacuoles containing glass; that although so large a percentage had ingested glass to some degree, yet only twelve per cent. formed more than one vacuole; and that only two per cent. were observed with more than three vacuoles containing glass. It is therefore evident that while glass is ingested, it is taken only in very small amounts.

Table II.

Experiments on Blue Glass

Table showing the results obtained in feeding *Dileptus* on powdered blue glass. In the five columns under the headings 0–4, are indicated the number of individuals which formed 0, 1, 2, 3, 4, or more vacuoles containing blue glass, respectively, during the twenty minutes of the experiment. Glass was ingested by 89.3 per cent. but in only 12 per cent. was there more than a single vacuole containing glass formed.

Experiment Number.	Total Number of Dilepti.	Number of Vacuoles Containing Glass.				
		0	1	2	3	4 or More.
1..........	15	4	8	2	1	0
2..........	14	3	10	1	0	0
3..........	15	0	10	3	1	1
4..........	15	0	15	0	0	0
5..........	15	1	14	0	0	0
6..........	15	3	9	2	0	1
7..........	16	0	16	0	0	0
8..........	15	0	14	1	0	0
9..........	15	4	11	0	0	0
10..........	15	1	9	3	1	1
Average.....	15	1.6	11.6	1.2	.3	.3
Per cent.....		10.7	77.3	8	2	2

Table III. contains the results obtained with india ink. It shows that almost ninety-five per cent. contained at least one vacuole with ink particles in it, and that only twenty-seven per cent. had formed a second vacuole during the entire twenty minutes. We can conclude, therefore, that ink is eaten in small amounts by the great majority of specimens of *Dileptus*, but that only relatively few form more than two vacuoles containing this substance.

TABLE III.

EXPERIMENTS ON INDIA INK.

Table showing results obtained in feeding *Dileptus* on India ink. In the columns under the headings of 0–4, are indicated the number of individuals which formed 0, 1, 2, 3, 4, or more vacuoles each, containing India ink, during the twenty minutes of the experiment. Ink was ingested by more than 94 per cent., but less than 28 per cent. formed more than a single vacuole containing ink.

Experiment Number.	Total Number of Dilepti.	Number of Vacuoles Filled with Ink.				
		0	1	2	3	4 or More.
1	15	1	11	2	0	1
2	15	0	12	2	1	0
3	15	0	11	1	2	1
4	16	1	13	2	0	0
5	17	2	11	3	1	0
6	15	3	12	0	0	0
7	15	0	8	4	1	2
8	12	1	7	3	0	1
9	15	0	3	5	4	3
10	15	0	13	2	0	0
Average	15	.8	10.1	2.4	.9	.8
Per cent.		5.3	67.3	16	6	5.3

In the experiments on all the other inanimate substances mentioned earlier, results were obtained which are, in the main, in harmony with those presented in Tables I., II., and III. All these substances, with the possible exception of sand, were ingested by a great majority of the individuals used in the tests. Experimentation with sand was very difficult owing to the fact that it settles very quickly, and that it is also difficult to see. My notes on the few experiments made record only thirty-two per cent. as having fed on this substance. The results obtained in experiments with chalk are almost identical with those obtained with glass. In the experiments on starch there does not appear to have been as sharp a decline between the number forming only one vacuole and those forming three or four vacuoles each within the twenty minutes. In other words, the power of discrimination does not seem to be as well developed in regard to this substance as it is in regard to the others. These experiments on inanimate substances thus show quite clearly that *Dileptus* when hungry will ingest insoluble substances, but that usually only one vacuole is formed.

B. *Animate Substances.*

In comparison with the results obtained in the above-described experiments on inanimate substances, those obtained in experiments on living material stand out in sharp contrast. Experiments were made with most of the forms listed in Table VII. The results obtained with all of these forms are essentially the same. Those obtained with *Euglena, Colpidium,* and *Chilomonas* are presented in Tables IV., V., and VI. By referring to these tables it will be seen that *Euglena* was ingested by 95 per cent. of the dilepti tested, 89 per cent. forming three or more vacuoles, and 50 per cent. four or more; that *Chilomonas* was ingested by 98 per cent. of the dilepti tested, 70 per cent. forming three or more vacuoles; and that *Colpidium* was ingested by 99 per cent. of the dilepti tested, 84 per cent. forming more than one vacuole. It is consequently evident that these organisms are ingested in large numbers by the majority of the dilepti tested.

TABLE IV.

EXPERIMENTS ON *Euglena.*

Table showing results of ten experiments obtained in feeding *Dileptus* on *Euglena.* In the five columns under the headings 0–4, are indicated the number of individuals which formed, in twenty minutes, 0, 1, 2, 3, 4, or more vacuoles containing *Euglena. Euglena* was ingested by more than 94 per cent. and a second vacuole containing this flagellate was formed by 89.3 per cent. of the individuals tested.

Experiment Number.	Total Number of Dilepti.	Number of Vacuoles Containing *Euglena.*				
		0	1	2	3	4 or More.
1	15	1	0	1	4	9
2	15	0	0	1	3	11
3	14	0	0	1	5	8
4	12	1	2	0	4	5
5	16	0	1	2	2	11
6	17	0	1	3	2	11
7	15	0	0	1	5	9
8	15	0	0	0	3	12
9	16	6	3	2	3	2
10	15	0	1	3	2	9
Average	15	.8	.8	1.4	3.3	8.7
Per cent		5.3	5.3	19.3	22	58

TABLE V.

EXPERIMENTS ON *Colpidium*.

Table showing results obtained in feeding *Dileptus* on *Colpidium*. In the five columns under the headings 0–4, are indicated the number of individuals which formed, in twenty minutes 0, 1, 2, 3, 4, or more vacuoles containing *Colpidium*. *Colpidium* was ingested by 99 per cent. and 84 per cent. formed more than one vacuole.

Experiment Number.	Total Number of Dilepti.	Number of Vacuoles Containing *Colpidium*.				
		0	1	2	3	4 or More.
1..........	14	0	3	5	6	0
2..........	15	0	2	4	3	6
3..........	12	0	3	2	5	2
4..........	11	0	0	4	6	1
5..........	15	1	5	3	4	2
6..........	15	0	1	7	2	5
7..........	12	0	3	4	4	1
8..........	12	0	2	3	6	1
9..........	15	0	0	4	8	3
10..........	14	0	1	3	7	3
Average.....	13.5	.1	2	3.9	5.1	2.4
Per cent.....		.7	14.8	28.9	37.7	17.7

TABLE VI.

EXPERIMENTS ON *Chilomonas*.

Table showing results obtained in feeding *Dileptus* on *Chilomonas*. In the five columns under the headings 0–4, are indicated the number of individuals which formed 0, 1, 2, 3, 4, or more vacuoles containing *Chilomonas* in twenty minutes. *Chilomonas* was ingested by 98 per cent. of the dilepti tested and 93 per cent. formed more than one vacuole.

Experiment Number.	Total Number of Dilepti.	Number of Vacuoles Containing *Chilomonas*.				
		0	1	2	3	4 or More.
1..........	14	0	1	3	4	6
2..........	15	0	0	6	4	5
3..........	15	0	1	3	4	7
4..........	15	0	1	4	2	8
5..........	15	0	0	2	7	6
6..........	15	1	1	5	3	5
7..........	16	1	0	3	4	8
8..........	15	0	2	3	7	3
9..........	15	0	2	1	4	8
10..........	15	0	0	4	3	8
Average.....	15	.2	.8	3.4	4.2	6.4
Per cent.....		1.3	5.3	22.7	28	42.7

By comparing these results with those on inanimate substances as shown in Tables I., II., and III. it will be noted at once that the living material was ingested by a much greater percentage of the dilepti tested, and also that the average number of vacuoles formed during the twenty minutes of the experiment was almost three times as great in the experiments on living material as in those on inanimate substances. It will also be noted that when feeding on inanimate substances *Dileptus* tends to stop feeding after having formed one vacuole, but that when it is feeding on animate substances it does not. This clearly indicates some power of selection, for *Dileptus* refuses to take in useless materials while it ingests nutritive substances in large amounts.

Having thus observed that *Dileptus* can select between animate and inanimate substances, the question naturally arose as to whether there is any choice between different kinds of organisms. Many species of organisms were used in attempting to answer this question. The same methods were used as described above, but only the results obtained in observations on the actual process of feeding were recorded.

The results obtained in these observation, in so far as they pertain to the problem of selection, are briefly summarized in Table VII. By referring to this table it will be seen that *Dileptus* does

TABLE VII.

DISCRIMINATION BETWEEN DIFFERENT ORGANISMS.

Table giving results of feeding tests with Dileptus showing selection among living organisms.

I. Organisms Readily Captured.	II. Organisms Captured only Rarely.	III. Organisms Never Captured or Injured in Any Way
Euglena gracilis(?)	*Paramecium aurelia*	*Paramecium caudatum*
Trachelmonas	*Frontonia*[1]	*Frontonia*[1]
Amœba	Rotifers	*Euplotes*
Halteria	*Stylonychia*	*Nassula*
Urocentrum turbo	*Spirostomum*	
Chilomonas paramecium		
Colpidium		
Colpoda		
Stentor cœruleus		
Stentor polymorphus		

[1] Two races of *Frontonia* were used, the one being entirely immune to the attacks of *Dileptus*, and the other only partially.

not capture all organisms, but selects from among the different kinds of living organisms in accordance with the grouping shown. The basis for this grouping will be more readily understood after presenting a few observations on the mechanics of feeding and the function of the trichocysts.

4. OBSERVATIONS ON THE MECHANISM OF FEEDING.

Many detailed observations were made on the mechanics of the feeding process in *Dileptus* in the hope of ascertaining the nature of the power of choice which *Dileptus* has been shown to possess. A few of them, illustrating the various factors involved in the feeding process, are described below.

A. Euglena.

In making observations on the capture and ingestion of *Euglena,* a single starved dileptus was isolated in a minute drop of water on a glass slide. To this another small drop containing many euglenæ was added. The reactions were observed under a magnification of about 350 diameters. In numerous observations it was found that whenever a euglena came in contact with any part of the oral surface of the proboscis of the dileptus, it at once become motionless (Fig. 2, *a*), and remained so for a longer or shorter

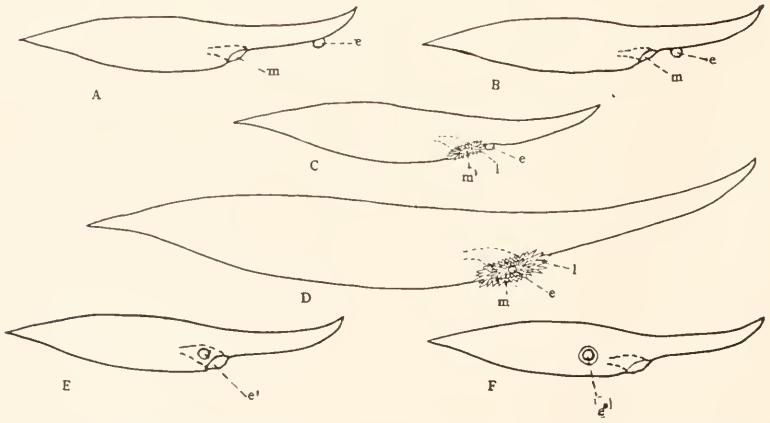

Fig. 2. Diagrammatic sketches illustrating the process of feeding. *A-F,* successive stages in process of ingesting *Euglena.* *e,* euglena paralyzed by trichocysts; *m,* mouth; *m',* mouth with protruding lips (*l*); *e',* euglena being engulfed; *e'',* euglena in food vacuole.

period, depending upon various conditions. It then reacted negatively with great vigor, and simultaneously or occasionally after a very brief latent period, it bulged out in the center and went through the typical euglenoid contractions with great vigor, sometimes holding the contracted form for two or three minutes. If, immediately after the first reaction, it failed to get out of the oral current which is continuously produced by the band of cilia on either edge of the flattened surface of the proboscis, it was carried to the mouth and engulfed (Fig. 2, *b*). If, on the contrary, the first reaction carried it outside the influence of the oral current, it began after a short interval to show some activity and soon recovered, unless it again came in contact with the proboscis. If this occurred, it was almost always carried to the oral region and engulfed.

The process of engulfing was quite extraordinary. Whenever the oral current carried a euglena to the mouth, the gullet, apparently owing to mechanical stimulation, protruded so that a mass of viscous protoplasm was exposed (Fig. 2, *c*). If any particle came in contact with this it adhered, and when this occurred the gullet was again drawn in carrying the particle with it (Fig. 2, *d, e*). In this process there was apparently some suction, for considerable water was always taken in with the solid particles (Fig. 2, *f*).

These observations were repeated on many favorable occasions, and, furthermore, are substantiated in the main by a similar observation recorded by Wrzesniowski ('70). Referring to the capture of a *Stylonychia* by *Dileptus,* Wrzesniowski says, "it tries by means of its proboscis to bring it down into its occasionally wide open mouth, whereupon the *protruding lips* seize so firmly upon the captured little animal that the latter is bitten in two." My observations agree with only the first part of this quotation; the idea of biting is quite contrary to the results of any observations which I have made.

B. ROTIFERS.

In the observations on feeding on rotifers described below, nine specimens were added to a small amount of water containing about twenty starved dilepti. Within two minutes all the rotifers were attacked. The dilepti appeared to sense (?) the rotifers while still at a distance at least equal to their own length. Sometimes as many as two or three of these ciliates were seen to gather around and attack a single rotifer. In these attacks the dilepti usually failed to capture the rotifers. Only in one instance was a rotifer actually observed to be captured and eaten. Although the feeding process rarely culminated successfully, this experiment afforded observations which are very instructive, as the following indicate.

In these observations each dileptus was continuously swinging its proboscis back and forth, and at the same time revolving on its longitudinal axis. Thus it struck the rotifers, now with the aboral side, now with the oral side of the proboscis, and the corresponding differences in the reactions of the rotifers were most striking. The rotifer in question is one which attaches itself quite securely to the wall of the dish. It also elongates and contracts from time to time without changing its location. When the aboral side of the proboscis of a dileptus came in contact with an elongated rotifer it contracted but slightly (Fig. 3, *B*), if at all, but when the oral surface of the proboscis struck the rotifer it contracted completely

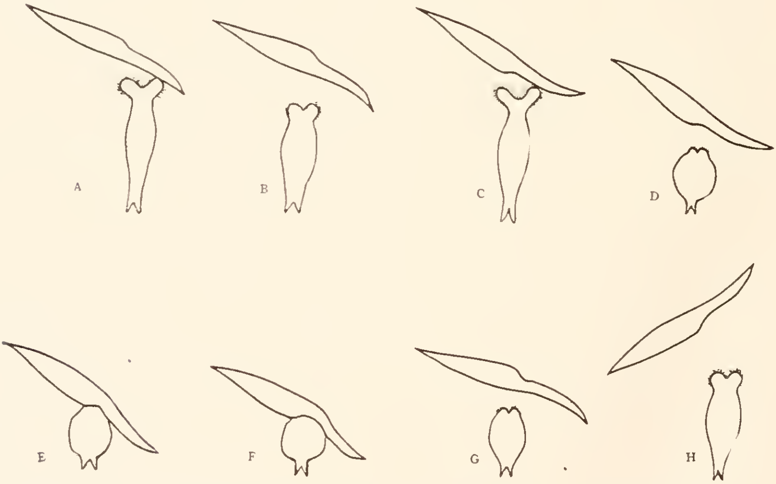

FIG. 3. Sketches illustrating the effect of *Dileptus* on rotifers. *A–H*, successive stages. Note differential effect, as seen by degree of subsequent contraction, of contact with aboral surface of proboscis as seen in *A* and *B*, and of contact with oral surface of proboscis as seen in *C* and *D*. For further explanation see text.

and vigorously, and often remained thus contracted for some little time (Fig. 3, *D*). The hungry little ciliates still persisted and an occasional individual was sometimes seen to succeed in getting the head of an attached rotifer far into its oral opening (Fig. 3, *F*), but a sudden contraction on the part of the attached rotifer invariably resulted in freeing the captive.

Thus when a rotifer comes in contact with the oral surface of the proboscis of a dileptus its reaction is extremely vigorous, while if the aboral surface of the proboscis touches the same rotifer little, if any, reaction is observed. The reaction resulting from contact with the aboral surface of the proboscis is just such as would be expected from a sight mechanical stimulus, but the violent reaction observed whenever the oral surface comes in contact with the rotifer is clearly of an entirely different nature. This difference must be in some way related to the difference between the oral and aboral surfaces of the proboscis. The essential difference between these two is the fact that the former contains trichocysts, while the latter does not. The difference in the reaction is probably, therefore, related to the action of the trichocysts.

C. COLPIDIUM.

The observations on *Colpidium*, like those on *Euglena* described above, were made under high magnification. In one of the many experiments two starved dilepti were isolated in a single drop of water and a smaller drop containing numerous specimens of *Colpidium* was added. The latter were so numerous that they were continually coming in contact with various parts of the dilepti. Some, consequently, frequently came in contact with the oral surface of the proboscis, as well as with various regions of the surface of the body. It was very apparent that those which came in contact with the oral surface of the proboscis were the only ones seriously affected. Whenever a *Colpidium* came in contact with this surface of the proboscis it at once became motionless and remained so for a very brief interval (Fig. 4, *A, a*). Then it suddenly became very active and swam away rapidly. Very often, however, with only a part of its body, for the part which came in contact with the proboscis bulged out and seemed to increase in volume, somewhat comparable to that which takes place when water is added to gelatine, but very much more rapidly. This mass was usually constricted off from the remaining part (Fig. 4, *B, b*), sometimes immediately, sometimes later. If this occurred immediately, the portion constricted off was carried to the gullet by ciliary currents and engulfed. Otherwise this portion was dragged along for a longer or shorter period by the active portion of the *Colpidium* (Fig. 4, *c, e*). Most of the *Colpidia* which were injured in this way soon died. Only a few of those which were isolated survived, and these had apparently lost only a small portion of their cytoplasm.

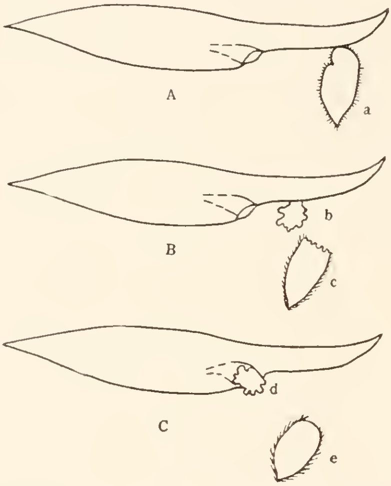

Fig. 4. Sketches illustrating the effects of the trichocysts of *Dileptus* on the infusorian *Colpidium. A, B,* and *C,* successive stages in the process of feeding. *a,* colpidium (motionless after contact with oral surface of proboscis); *b,* portion of colpidium (apparently cytolyzed and separated from major portion, *c*); *d,* cytolyzed portion being engulfed by dileptus; *e,* major portion of colpidium swimming rapidly away.

It seems evident from many observations like the above that it is the trichocysts of *Dileptus* which function primarily in the capture of *Colpidium.* The colpidia are first momentarily paralyzed and then excited to vigorous reaction, but as a rule only after a part of their cytoplasm has been in some way altered so that it disintegrates and *Dileptus* is enabled to feed on it in the same way that it does on other non-motile material.

The results presented seem to indicate that in *Dileptus* there are two distinct processes involved in feeding: (1) The capture of food and (2) the ingestion of food. In feeding on motile organisms both processes are involved, but when feeding on non-motile substances only the latter process is involved. When feeding on motile forms the prey is first paralyzed, and in many cases where this is only temporary the cytoplasm of the prey is locally seriously affected. In some forms that part of the protoplasm affected is constricted off, while in other forms the entire organism is affected. In this non-motile condition the prey is carried passively by the ciliary current to the buccal cavity and there ingested as described above. The trichocysts are evidently the structures which enable *Dileptus* to capture living prey and make the feeding process of this organism so complicated. The remaining part of the paper will be devoted to observations and discussion as to their nature and function.

5. TRICHOCYSTS.

The nature and function of trichocysts has long been a debated question and even today most authors admit that we know very little about them except in the case of one or two organisms which have been studied very extensively, and even here there is much controversy.

Mitrophanow ('04) maintains that the trichocyst consists of a viscid fluid contained in a cavity in the ectoplasm, whence it is expelled by a sudden contraction of the ectoplasm and stiffens to form a solid thread under the action of the water medium.

Schuberg ('05), however, denies this and maintains that the unexploded trichocyst is a spindle-shaped body with a fine hair-like process at its outer end which reaches to the pellicle, and that when it explodes this material forms into a fine thread-like sharp-pointed rod, often showing a cap-like swelling at one end.

Calkins in 1901 maintained that there are only two types which have been definitely made out—a rod-like form as in *Loxophyllum* and a spindle-shaped form as in *Paramecium*. He also stated that "when protruded from the body they are apparently of the same size and shape as when within the ectoplasm." In 1910, however, the same author wrote concerning trichocysts in general (p. 27), "when the organism is irritated the contents of the capsules are thrown out with considerable force and the poison which they contain is strong enough to paralyze any single-celled opponent."

Minchin ('12) maintained that "the nature and mechanism of (the peculiar) trichocysts still remains to be explained," but described as typical those forms found in *Paramecium* and *Frontonia*.

Concerning the function of trichocysts there seems to be even less known than there is about the structure. Jennings ('06) wrote that trichocysts "are usually supposed to be weapons of defense, but whether they really serve for defense seems questionable," and suggested that their discharge may be only an expression of injury—"a purely secondary, even pathological phenomenon, like the formation of vesicles on the surface of an injured specimen."

Mast ('09), however, showed clearly that in *Paramecium* the trichocysts have a definite protective function. He observed that the trichocysts of *Paramecium* are discharged in response to injury, produced by *Didinium*, and that as soon as these trichocysts come in contact with the water they form a mass having a firm jelly-like consistency which serves to force the enemy back mechanically, and frequently results in setting the victim free. Calkins ('10, p. 27) says that "sometimes they are used as weapons of offense as well as protective organs," and in another place he describes predaceous protozoa as "usually armed with offensive organs in the form of trichocysts which may be shot out from the surface of the body or carried javelin-like at the extremities of projectile tentacles."

A. Observations on the Nature and the Function of the Trichocysts of *Dileptus*.

Numerous experiments and observations were made on *Dileptus* to ascertain, if possible, the function as well as the structure of the trichocysts, all of which, as previously stated, are located on the oral surface of the proboscis. A description of a few of the more illuminating of these experiments will follow, but before considering these we may briefly recall a few of the results of the observations on feeding which have a bearing on this subject.

Euglena, it will be recalled, is paralyzed as soon as it comes in contact with the oral surface of the proboscis, and after a short latent period shows characteristic signs of injury. The violent contraction of the rotifers on every occasion when they come in contact with that portion of *Dileptus* provided with trichocysts gives definite signs of their effect. The observations on *Colpidium* show that the trichocysts not only paralyze this organism, but produce a cytolytic effect upon the protoplasm of the prey.

The following observations are presented in order to show more specifically the precise manner in which these trichocysts function.

a. Effect of Trichocysts on Paramecium bursaria.

In making observations on the action of the trichocysts of *Dileptus* on *Paramecium bursaria,* a single starved dileptus was isolated and added to a small drop of water on a slide containing four specimens of *Paramecium bursaria.* Nearly all the water was then drawn off, after which a cover-glass ringed with vaseline was applied. Two of the paramecia were lost, but the remaining two and the dileptus were confined in so small an amount of water and were so much compressed that they could move only very slowly, and never more than their own length from the others. Consequently all reactions could be observed very accurately. The dileptus, although so compressed that it was more than three times its normal width, continued to rotate on its longitudinal axis and its proboscis was consequently thrown from one side to the other. On several occasions the posterior end of the dileptus came in contact with one of the paramecia, making small indentations in it without any noticeable reaction on the part of the latter. When, however, it slowly reversed its position and the oral surface of the proboscis came in contact with the paramecium, a sudden discharge of trichocysts from the paramecium was observed, so dense as to force the dileptus away. The latter continued to rotate slowly, all the time removing the barrier of trichocysts by means of its ciliary action. The next time only the aboral surface of the proboscis came in contact with the paramecium and no reaction resulted. The third time the proboscis struck the paramecium it was at a slightly different spot and another discharge of trichocysts resulted from the latter. After some little time this again was cleared away, and a fourth attack occurred at about the same spot as the first, this time with an entirely different result. The paramecium reacted much more violently than previously and at the point of contact a noticeable bulging of the protoplasm occurred. The next attack was at a new spot, with the characteristic discharge of trichocysts. But the following one was at approximately the same spot as the preceding. The protoplasm this time bulged out and formed a large protuberance, even some of the zoöchlorellæ flowing out into it. After half an hour four such protuberances were observed, in all of which it was evident that the pellicle of the paramecium had given way at one small spot, and that the mass of protoplasm which flowed out formed a protuberance with only a narrow connection with the interior. After repeated attacks the paramecium disintegrated.

The other paramecium, meanwhile, had slowly moved toward the dileptus, and this afforded an opportunity to repeat the observations just described. The results obtained in these observations were essentially the same as those obtained in the first observation. The second paramecium, however, appeared to react more vigorously and it consequently escaped more of the attacks than the first, with the result that at the end of half an hour it had only two protuberances, whereas the first, as previously stated, had four. Apparently each attack on the part of the dileptus was just as powerful at the end of the experiment as it was at the beginning.

b. Effect of Trichocysts on Stentor cœruleus.

Perhaps the most instructive, at least the most spectacular, experiment concerning the action of the trichocysts of *Dileptus* is one which can be performed very simply as follows: A dozen or more dilepti are starved for two days; a large blue stentor is then introduced, and the scene of a veritable barbecue is soon presented. The dilepti collect about the stentor and can be seen to strike the latter with their proboscides (Fig. 5). The surface that comes in contact with the stentors in this reaction does not appear to be purely accidental, for it was observed that the oral surface of the proboscis came in contact almost without exception. At the point of contact the pellicle of the stentor gives way momentarily (Fig. 5, *D, d*), and a globular mass of protoplasm is extruded. This mass is soon constricted off (Fig. 5, *F, b*), and the wound apparently heals over at once, while the extruded protoplasmic mass is readily ingested by the dileptus (Fig. 5, *G, b*). Thus, now here, now there, the stentor gives up part of its protoplasm and each part is eaten by the little dilepti, which sometimes more than treble in size after feeding on this organism. Meanwhile the stentor

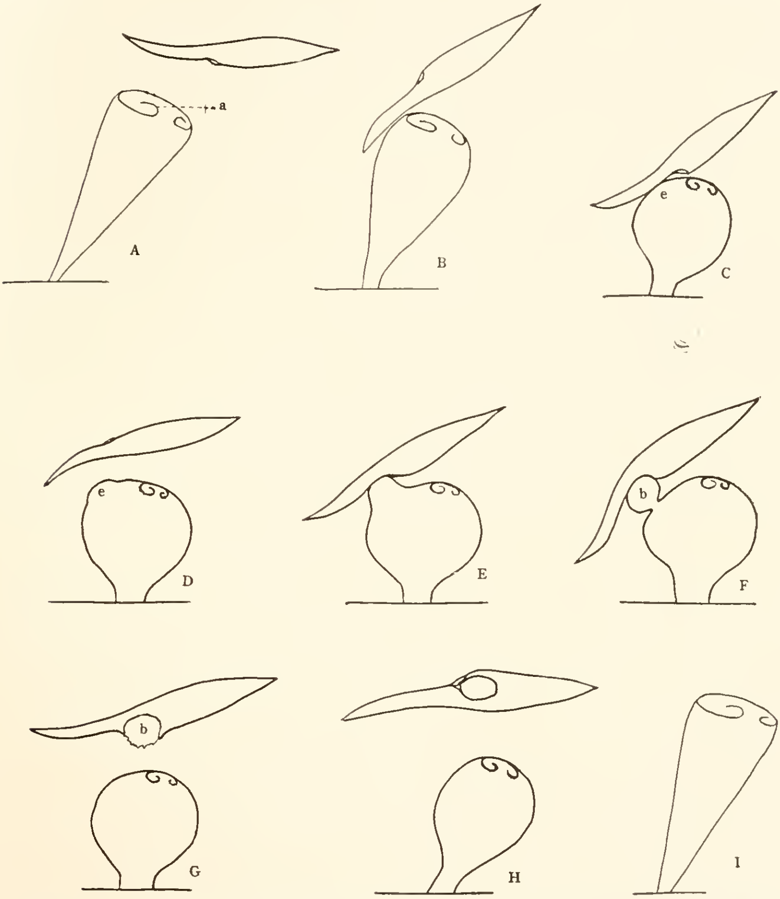

Fig. 5. Sketches illustrating effects of trichocysts of *Dileptus* on *Stentor cœruleus*. *A–I*, successive stages. *A*, normal stentor with epanded peristome (*a*). When stimulated as by contact with aboral surface of dileptus, the stentor contracts slightly as shown in *B*. If the oral surface of the proboscis comes in contact (*C*) the stentor contracts vigorously and its protoplasm in the region of contact (*e*) soon protrudes, *D, E,* and *F*. The injured area (*e*) soon gelates and the protruding mass (*b*) is constricted off, frequently being ingested by the dileptus (*G*). The stentor remains contracted for some time, but eventually, unless repeatedly attacked, it expands completely (*I*) and appears to be entirely normal.

continually shrinks in size, and unless it escapes before it is too badly injured it dies.

The trichocysts of *Dileptus* evidently affect the ectoplasm of *Stentor* and, as in the case of *Colpidium* previously described, result in a cytolytic action on the surface of the prey at the point of contact. This results in an outflowing of the inner protoplasm until the injured surface can again gelate in some manner, resulting in a new "pellicle."

If the observations just described are made under high magnification, it can be seen that the proboscis of dileptus never comes in actual contact with the body of the stentor. They are always separated by a space, at least equal to the sum of the lengths of their respective cilia. This would lead to the conclusion that the trichocysts of *Dileptus* are discharged through some little distance—that is, they are thrown out with some force.

c. Effect of Trichocysts on Paramecium aurelia.

Dileptus is normally unable to injure *Paramecium aurelia* in any way, but in one experiment several paramecia were seriously injured by two dilepti. In two instances, which were carefully observed, the paramecia appeared to be completely paralyzed, although only momentarily, immediately upon coming in contact with the oral surface of the proboscis. When the proboscis touched the paramecia they reacted vigorously and swam away, but not before they were injured. It was observed that they became much deformed soon after the attack, doubling on the point that had been injured to such an extent that they assumed the form of a horse shoe. One of these paramecia was attacked a second time while in this semi-quiescent condition and was successfully engulfed. The other one was isolated on a hollow ground slide and after about an hour began to swim about, gradually losing its deformity. On the following day it appeared to be normal. The ectoplasmic pellicle of *Paramecium aurelia* is probably of such a nature that it prevents any cytolytic action resulting from the trichocysts. The injurious effect of these structures seems to be due to the production of a definite wound at the point of contact.

d. Effect of Trichocysts on Spirostomum.

When a spirostomum is attacked by a dileptus it contracts vigorously as soon as "stung" (Fig. 6, *A, B*). This usually produces a violent reaction which serves to get it out of reach of the dileptus

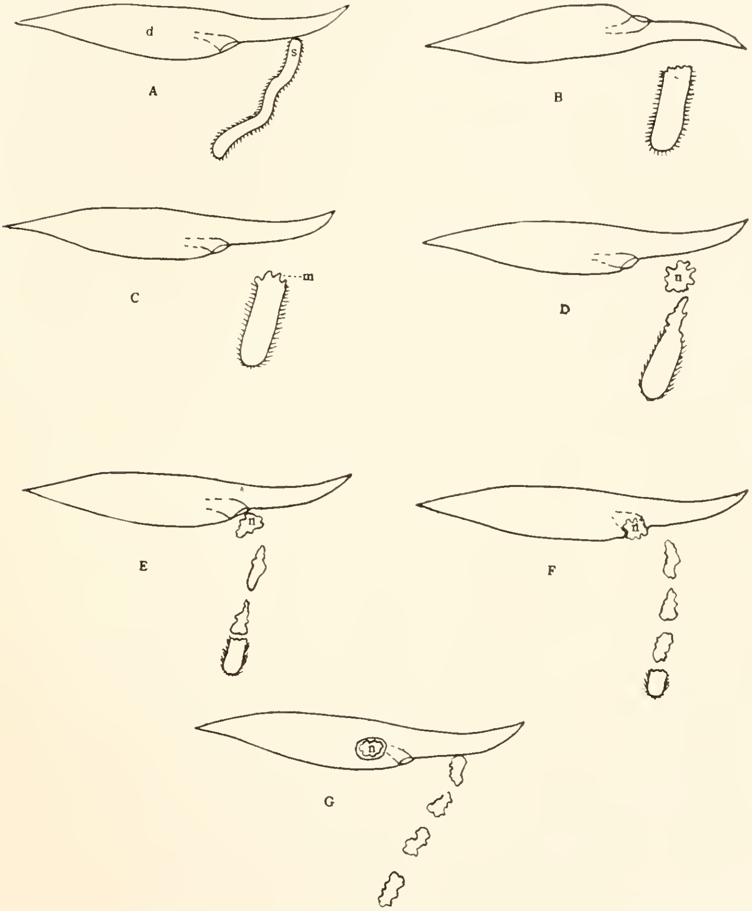

FIG. 6. Sketches illustrating the effect of trichocysts of *Dileptus* on *Spirostomum*. *A–G*, successive stages in process of feeding. When dileptus (*d*) comes in contact with spirostomum (*s*) as in *A*, the latter contracts vigorously and remains momentarily motionless (*B*). Cytolysis begins at area of contact (*m*) and as the spirostomum reacts negatively, swimming rapidly away, the cytolytic process continues, as in *D*, *E*, and *F*. Meanwhile dileptus has engulfed one or more masses of the disintegrating spirostomum (*n*) as shown in *D–G*.

and thus prevents a second attack. If it is attacked a second time, the result is extensive disintegration of the protoplasm around the point of contact (Fig. 6, *C, m*). If this point is located at one end of the spirostomum, the opposite end swims rapidly away, leaving behind a trail of disintegrating protoplasm (Fig. 6, *E–G*). When any part of the protoplasm of this organism begins to disintegrate, the cytolysis, once begun, progresses rather rapidly until the whole organism has disintegrated. This effect is in contrast with that obtained in *Stentor,* in which an attack produced only local and partial disintegration. Apparently the protoplasm of *Spirostomum* does not possess the power of gelation as observed in *Stentor,* and thus the cytolytic action continues until the organism is disintegrated (Fig. 6, *G*).

The results obtained in numerous other observations made on various other organisms are all in harmony with those which have been described. All these observations seem to show conclusively that the trichocysts discharged by *Dileptus gigas,* first temporarily paralyze the prey, then produce a period of increased activity in the nature of a negative reaction on the part of the prey, and simultaneously effect a cytolytic action at the point of contact.

B. OBSERVATIONS ON THE STRUCTURE OF THE TRICHOCYSTS OF *Dileptus*.

Numerous specimens of *Dileptus* were fixed during various stages in the process of feeding and many different methods of fixation and subsequently staining were employed in an attempt to ascertain the structure of the trichocysts. Before they are discharged the trichocysts can be clearly seen in all properly stained specimens (Fig. 7). They are found, as previously stated, in a band on the oral surface of the proboscis. When stained they appear as elongated bodies (Fig. 7, *t*), which do not show any definite internal morphological structure, but appear to be more or less granular, and if stained at all, are always stained deeply. Fig. 7 shows the relative number, size, and shape of the trichocysts as seen in 4 μ sections (*A–D*), and in a total mount (*E*). In favorable specimens they can be seen in the living animal, where they appear as colorless, rather transparent bodies which change shape as the animal twists and turns. They have been seen to become almost spherical in shape when under pressure from the coverglass.

In all my work it has been impossible to observe any structures or formed elements of any kind which could be identified as trichocysts or their contents outside the body of *Dileptus*. The safranin

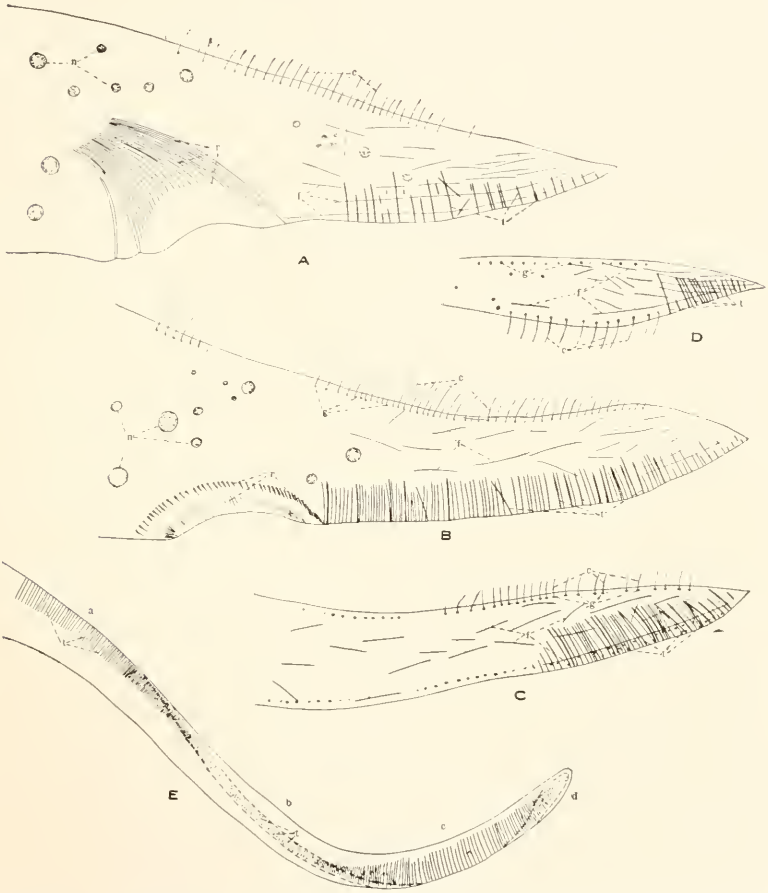

Fig. 7. Camera lucida drawings from preparations fixed in Schaudinn's fluid and stained with Fe-Haem. (*A–D*) or with acid borax carmine (*E*). *A*, *B*, *C*, and *D* are 4 μ serial sections of a proboscis. Note size, shape, and number of trichocysts (*t*); the pharyngeal rods (*r*); cilia (*c*); basal granules (*g*) "distributed nucleus," some of which are designated as *n*; and the contractile, fibrillæ (*f*). *E*, proboscis of dileptus, slightly twisted; from an entire mount. Note relative number and position of trichocysts (*t*), forming a band on the oral surface of the proboscis. Due to the turn in the proboscis, the trichocysts are seen from side view at *a* and *c* and from end view at *b* and *d*.

method of staining "intra vitam," which demonstrates so clearly the poisonous threads of the nematocysts of the common freshwater hydra, was also tried, but gave only negative results.

We would maintain, then, that the contents of the trichocysts of this organism do not have a morphological structure after they are discharged, such as the trichocysts of *Paramecium* and *Frontonia* have. It would appear that the trichocysts of *Dileptus* are more like elongated sacs of toxic fluid, which collapse upon discharging the fluid.

If it is true that these structures found in the proboscis of *Dileptus* are bags filled with a poisonous fluid, it is evident that the term trichocyst (hair-sack) is not exactly applicable, and in order to be more exact the term toxicyst (poison-sack) might be employed.

C. Summary of Observations on Trichocysts.

The foregoing observations and experiments show that the trichocysts of *Dileptus* are the structures which this organism employs in capturing food. They have the power to paralyze some organisms, to bring about the cytolysis of others, and to cause a vigorous reaction in almost all infusoria. Organisms like *Paramecium* and *Frontonia* are probably protected against the ordinary attacks of *Dileptus* by their own protective trichocysts. Organisms like *Euplotes*, which are provided with a lorica, form another class of infusorians which appear to be protected against the trichocysts of *Dileptus*. Certain species of *Stylonychia* are known to possess a heavy cuticle resembling a lorica, and it is perhaps for this reason that these organisms were but rarely observed to fall prey to *Dileptus*. The great majority of ciliates seem to fall prey to *Dileptus*, either owing to the paralyzing effect of its trichocysts or to the cytolytic action of these structures.

6. THE MECHANISM OF SELECTION OF FOOD IN *DILEPTUS*.

There appear to be two distinct mechanisms by which selection of food is brought about in *Dileptus*. (1) The rejection of inorganic particles, as shown in Tables I., II., and III., is evidently due to the effect of the physiological state, which serves to prevent the organism from ingesting more. (2) The purely chemical and physical properties of the trichocysts of *Dileptus* seem to determine very largely the nature of the food which this organism ingests. If the trichocysts are able to bring about cytolysis of the protoplasm of an organism, or even to completely paralyze it for a time, that organism is "selected" as food. This relation between the protoplasm of the prey and the trichocysts of *Dileptus* is the important factor in determining whether or not *Dileptus* "selects" it as food.

In *Dileptus* the former mechanism seems to play but a small rôle. Because of its natural habits this ciliate deals almost exclusively with living organisms. As previously stated, *Dileptus* thrives only in "relatively pure" and quiet water in which there are but few inorganic particles in suspension. Its habit of continually swimming serves admirably to keep it off the substratum, and we can readily comprehend that motile organisms are almost the only substances from which it has normally to select. We can safely conclude that much of the power of selection of food in *Dileptus* resides in the peculiar properties of its trichocysts.

7. SUMMARY.

1. *Dileptus gigas* normally feeds on living organisms, but under certain conditions it ingests inanimate particles.

2. It discriminates between living organisms and inanimate substances, ingesting the former in large amounts, while the latter are only sparingly ingested.

3. *Dileptus* selects from among different kinds of organisms, eating some with great readiness, while others are rarely ingested.

4. It captures its prey by means of trichocysts which either paralyze the prey, *e.g., Euglena,* or bring about cytolysis of all or part of the protoplasm of the prey, *e.g., Colpidium* and *Stentor.*

5. The trichocysts are probably of a liquid nature, highly toxic, with specific cytolytic properties.

6. The trichocysts of *Dileptus* are used for the purpose of capturing food.

7. Selection of food in *Dileptus* depends on two factors: (*a*) The physiological state of the organism itself, which appears to determine whether a substance shall be ingested in large or small amounts, and (*b*) the chemical properties of its trichocysts, which

determine in large measure whether any living organism can or can not be successfully captured.

8. Specialized structures as, for example, the trichocysts of *Paramecium* and the lorica of *Euplotes*, serve as protection against the attacks of *Dileptus*.

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BIOLOGICAL BULLETIN

THE GROWTH OF THE PAINTED TURTLE.

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As Agassiz (1857) pointed out, the growth of turtles is exceedingly slow. After comparing turtles of different sizes, Agassiz concluded that the eastern painted turtle (*Chrysemys picta*), after the eggs from which it hatched were laid, attained about the following lengths after one year: 26.5 mm.; 2:42; 3:51; 4:54; 5:59; 6:66; 7:72.5; 8:74; 9:77; 10:80; 13:92; 24:121. He affirms that this turtle does not lay eggs until it has attained an age of ten to eleven years. Lucas (1922) states that there are authentic records of tortoises that have lived to be one hundred and fifty years old. Barney (1922) has given a very careful account of the growth and breeding of the diamond-back terrapin when reared under cultural conditions in pens. He found that when domestic terrapins are fed during the winter, egg production occurs as early as the fourth year of age, but usually begins in the fifth or sixth year. Terrapins may reach a length of 130 mm. to 150 mm. in four years when fed in winter, and in six to seven years when allowed to hibernate. The maximum growth in length recorded for one year was 81 mm., and for two years 104 mm.

Since 1919 the writer has had opportunity to study the growth of the western painted turtle.[1] At various times, 406 turtles were marked with aluminum tags and immediately released in University Bay, Lake Mendota. These were measured when they were released and were caught at intervals and measured again. In this way the rate of growth was determined. The portion of the bay behind the bar, where the turtles were studied, is nowhere more than 1.5 meters in depth, has a soft, muddy

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[1] Ruthven's (1912) *Chrysemys belli* Gray and *C. cinera* (Bonnaterre) appear to intergrade in this locality, but a majority of the individuals resemble the former.

Table I.

List of Records of Growth of Turtles.

Abbreviations refer to months, from January to December, in the following order: J, F, M, Ap, My, Ju, Jl, Au, S, O, N, D, and indicate when each turtle was tagged and released.

Time Elapsed.			Length.		Gain.
Years.	Months.	Days.	Begin.	End.	
0	11	18	Jl 40.0	62.5	22.5
0	0	17	Jl 42.5	51.0	7.5
0	0	16	Jl 47.5	52.0	4.5
1	9	22	S 51	84.0	33.0
1	9	29	S 51	85.0	34.0
0	0	19	Jl 54	58.0	4.0
0	1	4	Jl 54	62.2	8.2
0	11	18	Jl 54	73.3	19.7
2	2	9	Jl 54	84.0	30.0
0	11	20	Jl 58.0	77.5	19.5
1	9	15	S 59	77.0	18.0
1	11	6	S 59	83.0	24.0
0	1	11	Ju 60	68.3	8.3
0	0	27	Jl 60	61.0	1.0
0	0	5	Jl 60	60.5	0.5
0	0	7	Jl 60	61.0	1.0
0	2	11	Jl 60	73.3	13.3
1	2	4	Jl 62	87.5	25.5
0	11	18	Jl 64	82.0	18.0
0	11	18	Jl 65	78.0	13.0
0	1	4	Jl 65	73.0	8.0
2	11	14	S 67	94.4	27.4
0	1	4	Jl 68	71.3	3.3
1	0	2	Ju 69	83.2	16.2
0	11	11	Jl 70.5	90.8	20.3
1	1	26	Jl 70.5	97.0	26.5
1	1	16	Jl 74	85.0	11.0
0	0	19	Jl 77	80.5	3.5
0	0	23	Jl 77	83.0	6.0
1	2	5	Jl 77	96.0	19.0
0	11	17	Jl 78.0	98.0	20.0
1	2	10	Jl 78	98.0	20.0
0	11	13	Jl 81.0	89.7	8.7
3	11	19	Au 82.0	95.8	13.8
1	5	16	F 87.0	91.0	4.0
0	2	18	Jl 89.5	91.0	1.5
0	11	17	Jl 89.5	92.3	2.8
1	2	21	Ju 90.0	108.2	18.2
0	3	3	Ju 90	94.5	4.5
0	7	14	My 91.0	92.0	1.0
1	2	4	My 91.0	106.0	15.0
1	9	27	S 95.0	97.3	2.3
0	1	3	Ju 97.0	98.0	1.0
1	0	3	Ju 97.0	99.6	2.6
1	2	0	My 99.0	101.5	2.5
1	0	6	Ju 99.0	102.0	3.0
1	1	5	My 100.0	103.5	3.5

Table I, *Continued*

Time Elapsed.			Length.		Gain.
Years.	Months.	Days.	Begin.	End.	
0	11	23	Jl 101.0	110.2	9.8
1	0	2	Ju 101.0	111.0	10.0
1	2	4	My 103.0	106.7	3.7
2	2	10	My 103.0	109.7	6.7
2	11	22	S 103.0	113.5	10.5
0	1	0	Jl 103.0	103.0	0.0
0	10	18	Ju 104.0	107.0	3.0
1	11	27	S 105.0	108.9	3.9
2	0	6	S 105.0	109.5	4.5
1	2	11	My 105.0	110.0	5.0
1	2	0	Jl 105.0	110.0	5.0
1	1	25	My 108.5	111.0	2.5
1	9	20	S 112.0	119.0	7.0
1	2	7	My 112.0	112.2	0.2
1	1	5	My 112.0	114.0	2.0
0	11	18	Jl 113.0	123.2	10.2
0	11	14	Jl 113.0	115.0	2.0
1	2	11	My 114.0	118.5	4.5
1	2	4	My 116.0	117.3	1.3
1	0	6	My 118.0	120.0	2.0
1	2	4	My 120.0	123.0	3.0
0	11	10	Jl 121.0	128.7	7.7
1	1	22	My 121.5	123.0	1.5
1	2	4	Jl 127.0	130.5	3.5
0	9	0	Ju 127.3	133.5	6.2
0	0	16	My 129.0	129.0	0.0
2	1	4	Au 130.0	137.0	7.0
4	0	6	Au 130.0	139.0	9.0
2	1	4	S 130.0	136.0	6.0
0	2	11	Jl 131.0	131.5	0.5
0	9	10	Jl 131.0	131.5	0.5
1	1	27	My 132.0	132.0	0.0
2	2	4	My 132.0	135.5	3.5
1	2	6	My 134.0	139.0	5.0
0	1	1	Jl 140.0	140.0	0.0
2	2	3	Jl 140.0	144.0	4.0
1	2	26	Ju 140.0	143.3	3.5
1	2	0	My 142.0	144.0	2.0
1	1	20	My 142.0	142.0	0.0
0	11	13	Jl 148.5	150.0	1.5

bottom, and maintains a vigorous growth of aquatic plants. An abundance of food was therefore available.

The records are summarized in Tables I. and II. It will be noted that some individuals grew very rapidly during a few days, and that other individuals of about the same size increased little or not at all during a considerable period of time. Such differences are probably correlated with the shedding of the dermal plates of the shell, growth being rather rapid immediately after the plates are lost.

TABLE II.

GROWTH OF TURTLES OF DIFFERENT LENGTHS.

Length in *mm.*	Number of records.	Average Rate of Growth; *mm.* Per Year.	Estimated Average Weight; Grams.	Estimated Average Weight Increase; Grams Per Year.	Percentage of Increase.
40– 50	2	32.7	19	13.8	73
50– 60	10	17.0	36	11.1	31
60– 70	12	16.7	50	12.7	25
70– 80	8	19.0	68	15.9	25
80– 90	3	4.2	106	5.2	5
90–100	11	6.0	134	8.5	6
100–110	13	3.5	170	5.7	3.3
110–120	8	3.1	233	6.3	2.7
120–130	6	4.2	243	8.2	3.4
130–140	8	1.5	310	3.4	2.4
140–150	6	1.6	362	3.9	2.7

Table II. shows that a turtle nearly doubles its length and weight during the second year of its life. After twelve years it would be about 135 mm. long and the growth rate would have decreased to about one thirtieth of that during the first two years. An ordinary adult turtle measuring 150 mm. in length is, using the data here presented as a basis for computation, about twenty-five years of age (since the eggs from which it hatched were laid). The largest turtle measured from Lake Mendota was 170 mm. in length. It was perhaps fifty years of age.

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A NOTE ON THE TOXICITY OF ACIDS FOR MOSQUITO LARVÆ.

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Interest in the resistance of mosquito larvæ to various physical and chemical reagents has been largely, if not solely, directed toward the discovery of practical methods for the eradication of the mosquito. A large amount of literature dealing with this phase of the subject is now available. The effects of various solutions of salts on mosquito larvae have also been rather extensively investigated and it has been found that the animals are able to withstand rather high concentrations of pure salt solutions (Mac Fie (1), Chidester (2), Sen (3), Sharma (4), etc.). It has recently been pointed out by MacGregor (5) that mosquito larvæ are able to live and develop in extremely high concentrations of acid, *e.g.*, acid of $P_H = 4.4$. In view of these last mentioned observations, it was thought advisable to test the toxicity of a series of acids of different concentrations for mosquito larvæ and the present paper embodies results obtained from such experiments.

The larvæ used in all experiments, *Culex pipiens*, were obtained in large numbers from small, stagnant pools usually found in uncovered containers. The entire culture as found was brought into the laboratory and tests carried out at the same time and with the same group of animals, all of which had presumably been under identical conditions. Both young and old larvæ were used and differences due to age noted. The animals were removed from the cultures by means of a wide-mouth pipette and transferred to a syracuse watch glass with as little of the culture medium as possible. In this way a large number of larvæ could easily be obtained for use in each experiment. Fifty to sixty animals were used in testing the effect of any concentration of reagents. The chemicals used (10 c.c.) were put into covered syracuse watch glasses; the larvæ were quickly injected into the solution and then observed until dead under a binocular microscope. The fatal exposure was taken at that time when approximately one half of the larvæ were killed, *i.e.*, when movements of the heart and alimentary canal ceased. Many cultures were used in experiments and slight variations in their resistance were shown but in the following only average results will be given. It is, however, of some interest to note that in practically all the cultures from which larvæ were obtained the hydrogen ion concentration showed them to be neutral or slightly alkaline in reaction ($P_H = 7.0–7.4$). The chemicals used were, hydrochloric, acetic, oxalic, butyric, salicyclic and carbonic acids and mercuric chloride.

Fatal Exposures in Minutes to Different Strengths of Acids (Temp. 25° C.).

Normality of Acid.	HCl.	Oxalic.	Salicyclic.	Butyric.	Acetic.
0.5	9.5	....	....	....	....
0.2	42.0	....	....	....	....
0.1	74.0	39.0	....	72.0	191.0
0.01	293.0	52.0	48.0	1440.0	1440.0+
0.001	1440.0+	1440.0	1200.0	1440.0+	1440.0++
0.0001	....	1440.0+	1440.0+	....	....

From the above table showing the length of life in minutes of larvæ in various strengths of acids it is evident that the animals are able to withstand abnormally high concentrations of acids for rather long periods of time. This remarkable resistance of mosquito larvæ is more strikingly shown when compared with that found for other forms—*e.g.*, Honda (6) found that the free-living nematode *Rhabditis elegans* withstood 0.01 normal HCl for 60 minutes, *Daphnia* for 23 minutes, tadpoles for 12 minutes and paramecium for 1 minute. (Personal communication to be published in *Journal of Experimental Zoölogy.*) MacArthur (7) found that Planarians are killed in a very short time by exposure to HCl of P_H 2–4.5. It has also been found by the author (8) that cysts of *Colpoda* withstand 0.001 N HCl for a strikingly long time.

That the hydrogen ion concentration is not necessarily the only factor in the toxicity of acids for larvæ is shown by comparing the effects of a saturated solution of CO_2 in H_2O of a P_H of approximately 3.7 with a solution of HCl of the same P_H value.

Larvæ in the CO_2 solution become motionless almost at once and the movements of heart and alimentary canal also quickly cease, while in HCl of the same P_H value they are apparently unaffected for over 24 hours. The more rapid penetration rate and mode of action of CO_2 as pointed out by Jacobs (9), doubtless account for the differences observed in the effect of the two reagents.

It is also of much interest to know in what manner the acids kill the animals, whether they enter the chitinous covering or enter by the mouth or anus through the alimentary canal. By using pupae, which are known not to eat nor to have external openings as in the larvæ, it is found that the acids do not kill them for many hours, considerably in excess of the lethal exposure for larvæ. From this fact it seems reasonable to assume that the larvæ are killed by the entrance of the reagent orally rather than cutaneously. The present discussion, however, deals primarily with the resistance of the animals to the reagents rather than with their mode of killing. Younger and smaller larvæ, and these doubtless have thinner chitin, are killed somewhat more quickly than older individuals.

The general order of toxicity of the acids used for the larvæ is, salicyclic > oxalic > HCl > butyric > acetic. This series is strikingly similar to that found by Haas (10) for plants, by Collett (11) for protozoa and by Bodine (8) for cysts of *Colpoda*.

FATAL EXPOSURES IN MINUTES TO DIFFERENT PERCENTAGES OF $HgCl_2$ (TEMP. 25° C.).

Per Cent. $HgCl_2$.	Time in Minutes.
0.05	305.0
0.10	155.0
0.50	48.3
1.00	26.5
2.00	20.0

Mercuric chloride in various concentrations was used and here again the resistance of the larvæ is of considerable interest. The above table shows the effect of different percentages of this salt. Honda (6) found that the free-living Nematode *Rhabditis elegans* withstood 0.05 per cent. $HgCl_2$ for 60 minutes; *Daphnia*, 25 minutes and tadpoles, 5 minutes. Mosquito larvæ are about 5 times as resistant to $HgCl_2$ as the most resistant form used by this author. Sen (3), with other species of mosquito larvæ (*Ste-*

gomyia albopicta) found that the animals were killed at once in a 0.1 per cent. $HgCl_2$ solution. This difference in length of time of fatal exposure to this concentration from the present result is doubtless due to the differences in resistance of the two species as well as to the end point taken in the experiments. Sen evidently used cessation of body movement while in the present investigation cessation of movements of heart and alimentary canal were taken as the end point.

SUMMARY.

1. Mosquito larvæ (*Culex pipiens*) were found to be extremely resistant to rather high concentrations of various acids.

2. The order of toxicity of the acids used is, salicylic > oxalic > HgCl > butyric > acetic.

3. The chemicals seem to penetrate the animal orally and not cutaneously.

4. Animals withstand rather high concentration of $HgCl_2$, considerably in excess of that found for other organisms cited.

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THE AMŒBOID MOVEMENT OF DISSOCIATED SPONGE CELLS.[1]

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INTRODUCTION.

In 1907 H. V. Wilson discovered a very interesting case of regeneration in siliceous sponges from dissociated tissue cells. The same phenomenon was observed in Hydroids, *Alcyonaria.* and Asteriæ (Wilson, 1911), in fresh water sponges (K. Müller, 1911) and in calcareous sponges (Huxley, 1920).

Twelve years before Wilson's discovery Roux (1895) described a similar phenomenon. He found that the blastomeres of the frog egg, artificially separated at an early stage of segmentation, and placed in water a short distance apart, slowly approached one another until they came into contact. Roux called this phenomenon cytotropism to correspond with other tropisms known in the scientific literature. He regarded cytotropism as a special case of chemotropism. This opinion was based on theoretical considerations as no experiments were made to prove it.

The term cytotropism although not definitely accepted has been used in various text books and scientific papers. Apparently the sponges with their ability to form the conglomerates from the dissociated cells afford the best opportunity for a study of this phenomenon.

Wilson and Müller (l.c.) made their studies on the regeneration of sponges after dissociation only after the aggregates began to form. Prior to this they made no exhaustive study, and merely stated that the separated cells coalesce and form aggregates. They did not study the amœboid movement which leads to the coalescence of cells and which is the main purpose of the present investigation. The work was started in 1920 at the Marine Biological Station at Sebastopol (Black Sea) and continued in 1921 at Woods Hole and in the Zoölogical Laboratory of Columbia University.

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[1] Contributions from the Sebastopol Biological Station, Crimea, and the Marine Biological Laboratory, Woods Hole, Mass.

The writer desires to express his gratitude to Dr. F. R. Lillie, Director of the Marine Biological Laboratory at Woods Hole, for accomodations there and to Dr. T. H. Morgan, of the Department of Zoölogy, Columbia University, for the courtesy of extending to him laboratory privileges.

Species Used for Experiments.

The coalescence of cells after their dissociation has been observed in many species of sponges. It is probable that all sponges possess this ability, but the formation of dense conglomerates and the power to regenerate a new organism occur only in a few forms. The species tested by the writer are as follows: Black Sea sponges—*Halichondria grossa* Schm., *Petrosia clavata* B. Cor., *Reniera densa* Bowerb., *Reniera informis* Schw., *Esperella lorenzii* Sch., *Kowalevskiella gracilis* Swarc., *Spongelia* sp., *Sycon* sp.; Woods Hole sponges—*Microciona prolifera* Verr., *Cliona celata* Gr., and *Grantia* sp.

Upon dissociation the cells of these species are able to coalesce and to form aggregates, but the grade of the formation varies. The best forms are *Reniera informis*, *Reniera densa*, *Petrosia coriacea*, and *Microciona prolifera*. The formation of aggregates in these species requires less time, the aggregates are more strongly attached to the substratum and they quickly transform themselves into new sponges.

Unfortunately many of the microphotographs, drawings, and other data collected by the writer at Sebastopol were lost owing to unavoidable circumstances and therefore the present work deals chiefly with the experiments made on *Microciona prolifera* at Woods Hole.

Amœboid Movement of Dissociated Cells.

The suspension of dissociated *Microciona* cells, obtained by squeezing the sponge through bolting silk No. 20, consists of three classes of cells, each of which can be easily recognized. The most abundant are the archaeocytes, nonspecialized, reddish cells about 8 microns in diameter and loaded with granules. Two kinds of these cells can be discriminated: the endoplasm of the first contains red pigment granules, to which the red color of the sponge is mainly due; the second contains in addition to a less abundant red pigment many dark yellow-greenish granules. Both forms are able to put out rounded or sometimes elongated pseudopodia and to display an active amœboid movement.

The second class consists of spheroidal dermal cells differing in size from 8 to 3 microns in diameter. They move very slowly, putting out short rounded pseudopodia.

To the third class belong the collar cells or so called choanocytes. They are partially dedifferentiated having lost their collars but still possess a long slender flagellum which continues to vibrate for at least three hours.

The process of reunion of the dissociated elements of the sponge tissue consists of following stages; sinking of the cells and adhesion with one another, adhesion to the bottom, amœboid movement and coalescence of cells, movement and coalescence of aggregates. The process of the formation of the aggregates can be easily observed in a microaquarium or in a hanging drop on a hollow slide. In both cases the globular aggregates are formed within three to four hours, the difference being that in the microaquarium the aggregates strongly adhere to the glass, while in a hanging drop they remain floating.

Among the different tissue elements of sponge the archaeocytes are the most active; some of them may send out pseudopods within a few minutes after the sponge was squeezed. They begin to move as soon as they come in contact with the bottom. The coalescence may even occur while the cells are still in suspension.

The character of the amœboid movement of both yellow and red archæocytes is the same; the cells put out large hyaline pseudopods and creep in different directions. The inner granuloplasm of the cells appears to be more viscous than their surface hyaline layer. This can be easily observed when two cells come in contact with one another. When two archæocytes touch one another their external hyaloplasm spreads out from both sides of the line of contact and flows round their bodies. Sometimes one can notice how the hyaloplasm is pressed away from the contact line between the cells and surrounds their bodies.

Often the archæocytes lose small drops of hyaloplasm which remain behind them indicating the route of the cell. Another archæocyte passing the same way may wipe out these drops which coalesce with its protoplasm.

On coalescence the inner granular protoplasm of the archæocytes remains unmixed; the granuloplasm of various cells occupies a definite portion of the aggregate, while the hyaloplasm forms a common mass surrounding the whole group.

By pressing the coverglass under which the aggregates are lying or by violently shaking a dish containing aggregates the cells can be separated after which they are able to coalesce again.

The amœboid movement of the archæocytes is irregular; some of them move actively while the others remain motionless or creep very slowly over the bottom. There is no visible difference between sluggish and active archæocytes. Frequently after being immobile for 60 or 80 minutes the archæocyte becomes active and starts to move rapidly.

In many cases when two archæocytes coalesce the aggregate remains motionless for a few minutes after which it starts to move in another direction. The coalescence with a small dermal cell or with a choanocyte does not disturb its movement; it continues to move as if there was nothing in its route.

The small dermal cells move very slowly or not at all. The choanocytes do not display an amœboid movement but are able to displace themselves by means of their active flagella.

The ectoplasmic layer of the isolated archæocytes is fluid and sticky. The cells easily adhere to different objects which come in contact with them. The adhesiveness of their protoplasm can be strikingly seen in a suspension containing starch grains. Each aggregate formed in such suspension is surrounded with a ring of starch grains. This occurs because the starch grains adhere strongly to the cells; as the aggregates move and turn in different directions they become surrounded with grains with which they accidentally come in contact. When moving the cells are able to push along or to carry various foreign bodies which they meet in their route. In one observation a small aggregate was found strong enough to push a group of five starch grains each of which was larger than the aggregate itself.

The formation of the aggregate from dissociated tissue cells is due to the motility of certain archæocytes and to the stickiness of their outer layer of protoplasm. The various cells are uniformly distributed in the suspension and over the bottom of the dish; each active archæocyte moves back and forth over a definite area and therefore cleans up the corresponding portion of the bottom, collects all cells lying in its route and finally forms an aggregate.

A well formed 24-hour-old aggregate of *Microciona* has the form of a ball; its surface is smooth and a thin hyaline membrane can be noticed on its periphery. Under unfavorable conditions the aggregates are irregular in form and fail to form a membrane. They are then unable to undergo futrher transformation and to regenerate into a new sponge.

Direction of Cell Movement.

There arises a question whether the approaching and the coalescence of the cells are due to a special kind of chemotropism or "cytotropism" or whether their movement is chaotic and their approach is a matter of a pure accident. If there be a directive force one may expect that the cells will move towards several others which form the centers of the attraction. In other words one ought to be able to detect a definite directive movement.

The simplest way to study the direction of cell movement is to draw contours of cells at definite intervals and then project all the outlines on one surface and in this way to reconstruct the paths of the cells. Such an investigation was made with a camera lucida and a combination of the Zeiss objective E and eyepiece 6 (× 625). A microaquarium was filled with a dilute suspension of Microciona (1 gram of sponge per 200 c.cm. of sea water); a sufficient number of paper sheets were placed on the table at the level of the microscope stage and were pierced at two points. The holes made by the punctures enabled one to put the sheets exactly upon one another after they had been removed from the table. Special precautions were made not to disturb the lower sheets when the upper one was removed. The sketches were made every two minutes and often every minute. The observations lasted from 40 to 190 minutes. After this period the movements are so slow that the continuance of the observation was unpracticable. The temperature of the water during the observations varied from 19° to 21° C. The observations were repeated many times with different colonies of *Microciona;* in all cases the character of the movement was the same, the difference being only in the velocity and in the duration of the movement.

The examination of the paths of various cells shows that their movement is not at all directed towards one another or towards the group of cells. No one cell could be found which acts as a center of attraction for other cells. The coalescence occurs only when one moving cell happens to touch another one, and to stick to its outer layer. The path of the archæocyte is an irregular winding line; a typical case is shown in Fig. 1. The movement of this archæocyte was followed for 168 minutes. The points where coalescence with other cells occurred are indicated by crosses; the arrows show the direction of the movement. Other cells in the same field of view moved very slowly and passed only few microns; the aggregate was formed exclusively through the activity of the archæocyte.

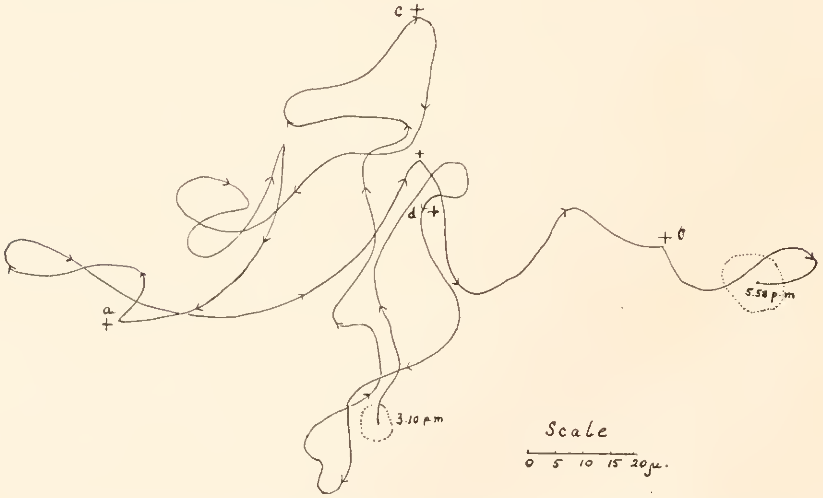

Fig. 1. The path of the archæocyte of *Microciona*; the line representing the movement of the center of the cell. The observation was made with camera lucida, Zeiss obj. E, and eyepiece 6. The outlines were drawn each two minutes.

This archæocyte travelled back and forth in different directions, approaching certain cells from which it withdrew. It coalesced first with the small dermal cell *d*, with the chaonocyte *c*, and with two archæocytes *a* and *b* all of which happened to lie in its route.

The velocity of the movement of the archæocyte is subject to many fluctuations. Even a fast moving cell sometimes stops and after a short period of rest resumes its movement. The retardation as a rule takes place when the cell changes its direction. The average velocity of an archæocyte measured in ten different experiments varied from .6 to 3.5 microns per one minute; the maximum velocity was as great as 20.0 microns per one minute. This maximum velocity was observed only twice. The distance travelled varied from 64 to 185 microns and active movement lasted from 40 to 168 minutes.

THE BEHAVIOR OF THE DISSOCIATED CELLS IN A COMPOUND SUSPENSION.

The cells of two different species of sponges mixed together coalesce only with cells of their own species. This ability of cells to discern the more foreign elements can be easily observed when two sponges of different colors are used. Wilson (1910) pointed out that the cells of *Microciona* mixed with those of *Lyssodendrix* and *Stylotella* form different clumps each apparently composed of the cells of same species; the clumps could be recognized by their natural colors. The same was found by the writer when a mixed suspension of *Reniera informis*, violet, Reniera densa, gray, and of *Microciona*, red, and *Cliona*, yellow, were tested. The aggregates formed in such emulsions were a violet, gray, red and yellow.

It is quite easy to distinguish the colors of the aggregates but as the color of a single cell is very slight it is impossible to distinguish the species while the cells are in suspension. In order to find how complete is the separation of the cells in a mixed suspension, one of the sponges before the experiment was fed with carmine, the other, with Chinese ink. The red and black granules ingested by the cells serve as definite marks indicating the species.

The suspension contained the cells of *Reniera informis* fed with carmine and that of *Reniera densa* fed with Chinese ink. The resulting aggregates lying close together consist exclusively either of carmine or of ink laden cells. The same occurs in a mixture of *Microciona prolifera* and *Cliona celata*.

Coalescence with the cells of another species never occurs even when the cells are artificially pressed together by centrifuging a mixed suspension. Examining after 24 hours such a clump of *Microciona* and *Cliona* one can find that in a common yellow mass of Cliona cells the Microciona had formed globular aggregates with marked membranes separating them from cells of the other species.

The formation of the aggregate in a mixed suspension does not go so completely as in a pure one; the aggregates are smaller and correspondingly more numerous than in the control dish. They are unable to undergo further transformation.

The coalescence of the dissociated tissue cells of sponges is apparently the same phenomenon as occurs in the extravasated blood cells of Arthropoda (Tait, 1918, Leo Loeb, 1920). In both cases the mechanical or chemical changes in the environment of the cells lead to its amœboid activity and to formation of aggregates of separated cells. In sponges the archæocytes *i.e.*, non-specialized elements, form aggregates which are able to regenerate a new organism. In blood cells the process does not go so far; the amœbocytes join in clumps and under favorable conditions can form a certain kind of tissue (L. Loeb). There is no indication of chemotropic or cytotropic stimuli in both cases and no such hypothesis is required to explain the results.

CONCLUSIONS.

The coalescence of separated sponge cells is the result of two factors: first, amœboid activity of the archæocytes, second a specific physical property of cell protoplasm which enables the cells to coalesce when they come into contact.

The coalescence of cells of two different species never occurs apparently because the physical properties of the protoplasm of the various species are different.

So-called cytotropism or a special kind of chemotropism does not exist in the cases studied.

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OBSERVATIONS AND EXPERIMENTS ON EUGLENOIDINA IN THE DIGESTIVE TRACT OF FROG AND TOAD TADPOLES.

ROBERT W. HEGNER.[1]

On a number of occasions during the past decade the writer has observed living flagellates of the *Euglena* type in the intestinal and rectal contents of frog tadpoles. They were always considered merely accidental inhabitants that had been ingested with the food of the tadpoles and were either immune to the digestive juices and were on their way through the intestine or had not yet succumbed to digestion. The observations and experiments described below, however, furnish evidence (1) that these flagellates are of widespread occurrence among the tadpoles of a number of species of frogs and toads, (2) that they are normal inhabitants of the intestine and rectum of tadpoles in the same sense that the better known protozoa, such as *Opalina*, are, (3) that they persist in starved tadpoles for many days, even after *Opalina* has disappeared, (4) that they retain their green color for a considerable period within the body of the tadpoles, (5) that they can be transferred in the trophozoite stage from one species of tadpole to another with food material, (6) that they differ in structure from any free-living or parasitic Euglenoidina heretofore described, (7) that they do not grow and multiply easily under ordinary culture conditions, (8) that certain free-living species of Euglenoidina are digested by tadpoles that do not digest the entozoic species, and (9) that certain tadpoles that were heavily infected with Euglenoidina did not become full-grown and undergo metamorphosis.

1. *A Comparison of Normal Tadpoles of Rana pipiens with Tadpoles Containing Large Numbers of Species A.*—The first series of observations, recorded in Table I. indicate that the

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euglenoid flagellate that I shall call Species A*, is a constant inhabitant of the tadpoles of *Rana pipiens* from Ithaca, N. Y., and that it may be a factor in the failure of certain specimens to grow and undergo metamorphosis normally. So far as I have been able to learn, this species has not been described although Alexeieff (1912) noted what may have been specimens of this form in the intestines of tadpoles. It is cylindrical and elongate with blunt anterior and posterior ends and decidedly metabolic. It ranges in length from 35 μ to 45 μ and in breadth from 4.8 μ to 6.4μ. The average length of ten specimens was 39.7μ and average breadth, 5.3 μ. There are three flagella almost as long as the body and about 50 green chromatophores, oval or circular in outline, lying in a single layer near the surface of the body. The nucleus is spherical and situated near the center of the body. Near the anterior end is a large spherical reservoir opening to the outside through a cytopharynx. At one side of the reservoir is the red stigma. Figs. 1 to 3 are of three specimens drawn with the camera lucida at a magnification of 1600 diameters. The specimen in Fig. 1 was living when drawn, in Fig. 2, killed and stained with iodine, and in Fig. 3, fixed in Schaudinn's fluid and stained by the iron-hæmotoxylin method.

The tadpoles were collected in artificial ponds at the fish hatchery of Cornell University at Ithaca, N. Y. My attention was directed to their study by Dr. G. C. Embody, who had noted the small size of specimens in one pond as compared with those in a neighboring pond only six feet distant. These ponds were about 4 feet square and 18 inches deep. One was well filled with algæ and the other was almost free from vegetation. Egg masses of *Rana pipiens* had been placed in these two ponds by Dr. Embody at approximately the same time, about April 20. The tadploes were collected seven weeks later (June 8). In Tables I. and II. are presented data regarding the differences (1) between the two sets of tadpoles and (2) between their rectal contents. The following points may be noted.

1. *Size.*—(Table I.) Although the two groups of tadpoles

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* After this paper was written I learned that Dr. D. H. Wenrich had also spent the summer of 1922 studying this organism. His work was largely devoted to its morphology and cultivation. He has given it the name Euglenamorpha hegneri. (Wenrich, 1923).

studied were of approximately the same age at the time they were examined they differed greatly in size. Those in the pond without algæ being on the average approximately twice as long in both

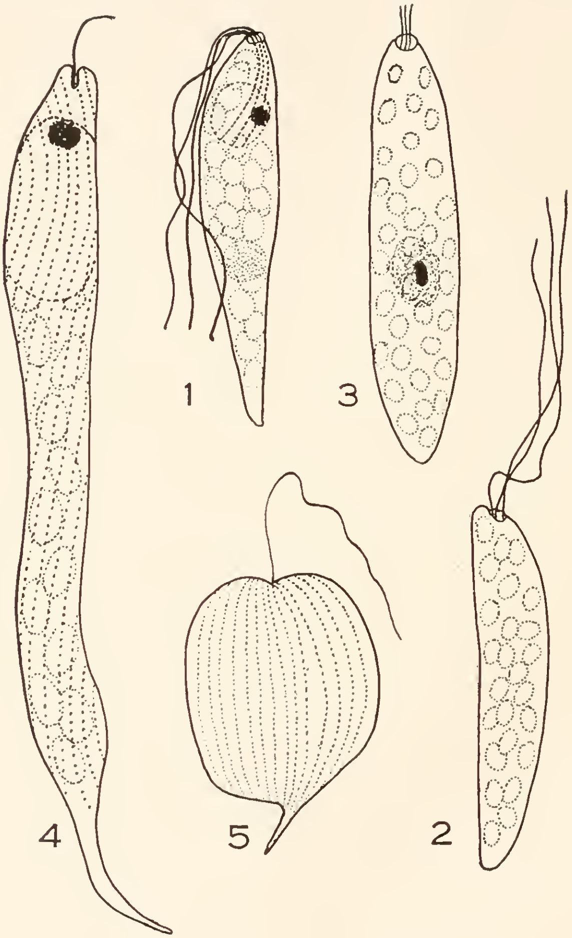

Fig. 1. A living specimen of a euglenoid of Species A showing characteristic shape, three flagella, reservoir, stigma, chromatophores and nucleus. × 1600.

Fig. 2. Species A as seen when stained with iodine. × 1600.

Fig. 3. Species A fixed in Schaudinn's solution and stained with iron-hæmotoxylin. × 1600.

Fig. 4. A living specimen of Species B showing flagellum, reservoir, stigma, and chromatophores. × 1600.

Fig. 5. A living specimen of *Phacus* found in the intestine of *Rana pipiens* tadpoles. × 780.

body and tail and in total length as the others. Every one of the "normal" tadpoles was larger than any one of the "dwarfs."

2. *Length of Intestine.* (Table I.) A high correlation between body size and length of the intestine was found. In almost every case the larger the body the longer was the intestine. The average length of the intestine of tadpoles from the alga pond was less than half that of the other tadpoles.

TABLE I.

MEASUREMENTS OF BODY, TAIL, INTESTINE AND RECTUM, IN MILLIMETERS, OF TEN SPECIMENS EACH OF "DWARF" AND "NORMAL" TADPOLES OF *Rana pipiens.*

	Length of Body.		Length of Tail.		Total Length.		Length of Intestine.		Length of Rectum.	
	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.
10 "Normals"	10–15	13.4	17–24	20.8	27–39	34.2	75–165	127.3	5–20	12.1
10 "Dwarfs"	6–11	8.1	8–15	11.2	15–25	19.3	30–92	58.3	3–10	5.7

3. *Length of Rectum.* (Table I.) The rectum of the tadpole is coiled in such a way that exact measurements are difficult to make. Those in the table are only approximate, but they show that in the "dwarfs" the rectum was only about half as long as in the "normal" tadpoles.

4. *External Evidences of Metamorphosis.*—All of the "normal" tadpoles exhibited rudiments of hind limbs. These measured from 2.5 to 0.5 mm. in length. Only one of the "dwarfs" possessed rudiments of legs and these were only 0.25 mm. long. The former had therefore progressed further in metamorphosis than the "dwarfs."

5. *Internal Evidences of Metamorphosis.*—Shortening of the intestine occurs during the metamorphosis of tadpoles. In judging this character, however, one must take into account the size of the tadpole. The shorter length of the intestine of the "dwarf" tadpoles is probably due to the small size of the animal rather than to a more advanced stage of metamorphosis. In both groups of tadpoles the rectum was well differentiated.

6. *Character of the Contents of the Intestine.*—The intestines

of the two groups of tadpoles differed in appearance, the difference being due apparently to the presence of algæ in the dwarfs and the absence of algæ in the normals. The intestines of the latter were filled with very fine particles of mud mixed with diatoms and minute organic debris; they were grayish in color, of uniform thickness, and smooth in outline. The intestines of the dwarfs, on the other hand, contained large pieces of algæ which appeared to divide the contents into separate masses, giving the entire intestine a patchy appearance; they were slightly tinged with green, and had an irregular outline due to the irregular distribution of the contents. The organisms noted in the intestines of both sets of tadpoles included a very few normal inhabitants of the rectum—*Opalina*, *Trichomonas*, and *Hexamitus;* at least 60 per cent. of both sets were inhabited by *Giardia agilis;* euglenoids were found in four of the dwarfs and one of the normals; and filamentous algae were present in considerable abundance in all of the dwarfs but in only one of the normals.

7. *Character of the Contents of the Rectum.*—The contents of the rectum of the normal tadpoles gave this part of the alimentary canal a grayish appearance, but in the dwarfs the euglenoids were so abundant that a distinct greenish color was produced. It was difficult to count accurately the number of specimens of the various organisms present. The method employed was to mix thoroughly on a slide the entire rectal contents in one drop of normal saline solution; spread this out under an 18 mm. square cover glass and count the number of organisms in ten separate fields in different regions of the slide using a 4 mm. objective and a 4 × ocular. Averages for each tadpole were then computed. Table II. gives the range and the average numbers of five different organisms. *Endamœbæ* and other forms were encountered but were not recorded. *Opalina*, *Trichomonas*, and *Hexamitus* were present in every tadpole and no significant differences in number were noted. The most conspicuous difference in the two groups of tadpoles was the presence of large numbers of euglenoids and of considerable amounts of filamentous green algæ in every one of the dwarfs and the almost complete absence of these in the normals. One or several euglenoids were found in the rectal contents of six of the normals after long searching and a little algæ was seen in one of them.

TABLE II.

COMPARATIVE NUMBERS OF SPECIMENS OF CERTAIN ORGANISMS IN THE RECTUM OF TEN SPECIMENS EACH OF "NORMAL" AND "DWARF" TADPOLES OF *Rana pipiens.*

The numbers were obtained by counting those in ten fields with a magnification of 520 diameters.

	Opalina.		*Trichomonas.*		*Hexamitus.*		Euglenoid Species A.		Filamentous Algæ.	
	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.	Present.	Abundant.
10 "Normals"	0.2–0.5	0.44	8–32	15.7	15–80	35.7	[1]		1	0
10 "Dwarfs"	0.1–2.0	0.67	3–48	13.6	2–40	23.6	4–60	16.0	8	2

The euglenoids were thus almost entirely restricted to the rectum of the dwarf tadpoles. They seemed to be in excellent condition; the pigment spot was bright red; the chlorophyll bodies were a brilliant green; and swimming activities and metabolic changes were apparently normal.

8. *Conclusions.* This comparative study of 10 specimens each from these two sets of tadpoles leads to the following conclusions:

(*a*) The euglenoid, Species A, is a constant inhabitant of the rectum of the tadpoles of *Rana pipiens* obtained from an alga pond at Ithaca, N. Y., and a rare inhabitant of the rectum of tadpoles of the same species from a neighboring alga-less pond. Infection with *Opalina*, *Trichomonas*, and *Hexamitus* was about the same in both sets of tadpoles.

(*b*) Although of approximately the same age the tadpoles containing many euglenoids were only about one half the size of the other set; and were less advanced in metamorphosis. The presence of these euglenoids may have been a factor retarding growth and metamorphosis.

II. *Effects of Starving the Host on the Persistence of Euglenoids of Species A in Tadpoles of Rana pipiens.*—After a comparison was made between tadpoles of *Rana pipiens* one set of which contained euglenoids in abundance and the other set few or none, it was decided to keep infected tadpoles in the laboratory and examine them at intervals to see if the infection persisted for any considerable period and if any changes in number, stage in life history, color, etc., would take place. Table III. gives the dates and results of examinations. One tadpole was used each day, the last one being examined on the twenty-fifth day. During this entire period the euglenoids persisted in the digestive tract, in numbers at least as great as in tadpoles examined on the date of collection. My method of counting (see p. 88) was not very accurate, but it seemed to me that the number of euglenoids was more numerous in tadpoles studied on later dates than at first. In the meantime the other protozoa common in the rectum of these tadpoles decreased markedly or disappeared entirely. No encystment was noted in any of the specimens and only one euglenoid was seen in division.

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[1] One or several specimens were present in 6 of the 10 tadpoles.

TABLE III.

NUMBER AND DISTRIBUTION OF EUGLENOIDS OF SPECIES A IN TADPOLES OF *Rana pipiens* COLLECTED JUNE 12 AND KEPT IN LABORATORY WITHOUT FOOD UNTIL DATE OF EXAMINATION.

For method of counting see text.

Date Examined.	Number of Days in Laboratory without Food.	Average Number per Field in Rectum.	Average Number per Field in Intestine.
June 17	5	Abundant	Many
June 18	6	8	24
June 19	7	10	3
June 20	8	23	Very few
June 21	9	37	Very few
June 22	10	19	Many
June 24	12	22	Many
June 25	13	Many	Many
July 4	22	Very few	Many
July 7	25	30	Very few

The euglenoids retained their normal free-swimming shape throughout the entire experiment, and were very active, swimming about by means of their flagella or undergoing rapid metabolic movements. No appreciable decrease was noted in the intensity of the green color nor in that of the eye-spot. This was probably due to the transparency of the ventral body-wall which allowed light rays to enter. A few days after the tadpoles were brought into the laboratory the rectum and intestine became almost free from food material and their contents could easily be seen through their walls. It was found that the euglenoids were usually most abundant in the rectum (see Table III.), almost as numerous in the first 10 mm. of intestine adjacent to the rectum, and fewer in number throughout the rest of the intestine. They were not, as a rule, distributed throughout the intestinal and rectal contents, but could be seen swimming about between these contents and the wall. Often they occurred in large groups thus giving a patchy green color to the digestive tract that could be discerned with the naked eye.

That these euglenoids also persist in tadpoles in nature was proved by the examination of three specimens collected from the same pond on June 24, *i.e.*, 12 days after the first lot were taken. These specimens all contained numerous euglenoids in both rectum and intestine; some of the euglenoids seemed to have become paler in color. The larval period of *Rana pipiens* is from 60 to 80 days but neither the tadpoles collected on June 12 and kept in the laboratory until July 7 nor those collected on June 24 and kept in the laboratory until July 9 increased in size nor advanced in development during this time, although they were about 75 days old and should have been undergoing metamorphosis. The presence of euglenoids may have been a factor in this retardation of growth and development.

III. *Infection of Rana pipiens Tadpoles with Food Containing Species A.*—Tadpoles of *Rana pipiens* in which there were a very few specimens of Species A were collected on June 24, 1922, when about 9 weeks old. On the following day 5 of these were fed on the recta from ten tadpoles of the same species in which there was an abundance of Species A. Previous examination of tadpoles from the same lot as those from which these recta were obtained gave an average number of sixteen euglenoids per field (see Table II., "dwarfs"). The 5 experimental tadpoles immediately began to devour the recta and all of the latter had been eaten by the following day and no specimens of Species A could be found in the dish. Uninfected (normal) and infected tadpoles were examined at intervals of one, three, five, nine, and twelve days. The results obtained are given in Table IV. It is evident from the increase from 0.6 per field to 4.4 per field that Species A has increased in the experimentally fed tadpoles and that this increase is due to the ingestion of specimens contained in the recta used for feeding purposes. Since these specimens were

Table IV.

Comparative Numbers of Euglenoids of Species A in the Rectum of Tadpoles of *Rana pipiens* Used as Food and in Control and Experimentally Fed Tadpoles.

Character of Tadpoles.	Number of Specimens.	Numbers per Field of Species A in Rectum.	
		Range.	Average.
Dissected for feeding	10	4–60	16.0
Controls	5	0.2–0.9	0.6
Experimentally fed	5	2–7	4.4

probably all in the free-swimming stage it seems certain that infection of *Rana pipiens* tadpoles can be brought about by the ingestion of active trophozoites. Such a method of infection might occur in nature since tadpoles will feed upon the dead bodies of other tadpoles, but this is probably not the usual method since 100 per cent. of infection has been observed in entire schools of young tadpoles, and, of course, there must be a resistant stage for maintaining the race through the winter and for infecting the first tadpoles in the spring.

IV. *Can Euglenoids of Species A be Cultivated Outside of the Body of ths Tadpole?*—The data presented above indicate that the euglenoids of Species A are regular inhabitants of the digestive tract of *Rana pipiens* tadpoles. The questions suggested by these results are; (1) are these euglenoids restricted to this habitat or can they also maintain a free-living existence; and (2) can other euglenoids known to be free-living be colonized in the rectum and intestine of tadpoles of this species. Two methods of answering these questions were employed: (1) an attempt was made to cultivate Species A outside of the body of the tadpole, and (2) tadpoles were fed on freeliving euglenoids and their digestive tract examined on subsequent days.

Euglenoids of Species A remained alive and active for at least 48 hours inside of the digestive tract that had been dissected out of tadpoles and kept in a small dish in water. Specimens also remained alive for 72 hours in material from the rectum and intestine under a sealed cover glass. Specimens that were dissected out of the digestive tract and placed in culture dishes did not live and multiply. This does not prove that they cannot maintain themselves outside of the digestive tract of the tadpole but indicates that they probably are restricted to an entozoic existence.

V. *Can Tadpoles of Rana pipiens be Infected with Free-living Euglenoids?*—It has been shown above that Species A can be transferred from one tadpole to another with the food, therefore if free-living species can live successfully in the digestive tract of these tadpoles it should be possible to bring about infection by including them with the food. On June 24, twenty-five tadpoles of *Rana pipiens* about nine weeks old were placed in a culture containing millions of small free-living euglenoids of a species possessing 2 short flagella, obtained from a large tub at the fish hatchery. In 18 hours these tadpoles had eaten every euglenoid in their medium. At this time five of these tadpoles and an equal number of controls were examined. The rectum of the experimental tadpoles was of a deep green color and the intestine also. Not a single specimen of the free-living euglenoids, however, could be found in any of these five tadpoles. The greenish color was due to minute chlorophyll bodies from 3 μ to 8 μ in diameter. These were the chromatophores of the disintegrated euglenoids. Chlorophyll bodies of this type were entirely absent from the five control tadpoles examined at the same time. In both experimental and control tadpoles there were present a few euglenoids of Species A and a few of a species to be described later as Species B. Experimental and control tadpoles were examined on the second day (5 specimens), fourth day (2), and eighth day (1). No euglenoids of the free-living type were discovered. The chlorophyll bodies gradually decreased in number. The conclusion reached is that an essential difference exists between euglenoids of Species A and those of this free-living type, the former being able to withstand the digestive juices of the host and to maintain themselves within the digestive tract, whereas this free-living species is unable to live in the same environment being killed and digested by the tadpole.

A second experiment was carried on at Baltimore during the month of September. Five tadpoles of the green frog that contained very few euglenoids were placed in a small amount of water in which there were thousands of a large reddish-colored euglenoid. One tadpole that was examined the following day contained many living euglenoids most of which were rounded and quiescent but a few of which were extended though sluggish. Besides these euglenoids there were large numbers of euglenoid chromatophores present proving that many specimens had been broken down within the digestive tract. The feces of the remaining four experimentally fed tadpoles were found to contain living euglenoids and these in the course of the next two weeks must have passed through the digestive tract of these tadpoles and been reingested again and again. One tadpole was killed and examined four days after feeding, another 13 days after feeding and the last two 20 days after feeding. The first two of these contained living euglenoids in both intestine and rectum, but the other two, which were prevented from reingesting their feces for a period of 7 days were entirely free from euglenoids. These results show that the free-living euglenoids used in this experiment had a higher degree of resistance to digestion within the tadpole than those employed in the first experiment but were unable to maintain themselves for a period of 20 days within the digestive tract.

A third experiment of a similar type was carried out with euglenoids obtained from the bladders of the bladderwort, *Utricularia*. I am indebted to my colleague, Mr. Bruce D. Reynolds, for calling my attention to this form and obtaining material for me. Plants obtained from a pond on the campus of the Johns Hopkins University were well supplied with these euglenoids although the surrounding water was entirely free from them. The numbers of euglenoids counted in ten bladders ranged from 8 to 510 in each, with an average of 215. Euglenoids were dissected out of 90 bladders and placed in a dish of water with three tadpoles of the green frog. The total number of euglenoids in this dish was thus about 20,000. All of these had disappeared from the water by the following day and none could be found in the fecal material in the dish. One tadpole was examined after two days and the other two after three days. No euglenoids were found in any of them. This type of euglenoid, therefore, is unable to withstand the digestive juices of the tadpole, although resistant to the secretions within the bladder of the *Utricularia* plant.

VI. *Euglenoids of Species A in Toad Tadpoles.*—A large number of toad tadpoles were collected from one of the large ponds at the fish hatchery on June 12, 1922. Their average measurements were: total length, 20 mm.; body, 8 mm.; tail, 12 mm.; hind legs, 3 mm.; intestine, 47 mm.; rectum, 9 mm. They were kept in the laboratory in a flat dish without change of water but with the addition of fresh water from time to time to compensate for evaporation. Some of the tadpoles developed fore legs and acquired the characteristics of the young toad within a few days; these apparently had reached a stage when no more food was necessary to bring this about. In most of them, however, growth and metamorphosis were inhibited by the condition of starvation to which they were subjected. Specimens were examined at intervals with the results presented in Table V. The following observations seem worthy of mention. (1) The incidence of infection with Species A was 100 per cent. (2) No encysted or dividing specimens were encountered. (3) Most of the euglenoids were swimming freely or undergoing metabolic movements; a few were spherical or pear-shaped. (4) The number of specimens was not diminished or increased by the starvation of the host. (5) Evidently Species A is a "normal" inhabitant of the rectum of toad tadpoles in this locality. (6) A decided decrease in the intensity of the green color of the chromatophores was noticeable in almost all specimens; some were pale green and others were almost colorless. This condition probably resulted from lack of light and may be contrasted with that observed in the case of specimens from starved tadpoles of *Rana pipiens*.

TABLE V.

NUMBERS OF EUGLENOIDS OF SPECIES A IN THE RECTUM OF TOAD TADPOLES COLLECTED ON JUNE 12, 1922, AND KEPT WITHOUT FOOD.

For method of counting see text.

Date Examined.	Number Examined.	Number of Days in Laboratory without Food.	Range in Number of Specimens per Field.	Average Number of Specimens per Field.
June 28	10	16	1–12	5.9
July 11	3	29	4–6	5
July 13	2	31	1–8	4.5

In the latter the abdominal body wall allows the light to penetrate to the intestine more easily than in the toad tadpole, which is characterized by the presence of dense black pigment. The nutrition of the euglenoids in the toad tadpoles thus becomes almost entirely by absorption whereas in the tadpoles of *Rana pipiens* it is still partly holophytic. It is interesting to note that Species A is sensitive to light, congregating on the side of the slide toward a north window and moving from one side of the slide to the other, a distance of 16 mm. in about 20 minutes, when placed opposite this window.

VII. *Infection of Toad Tadpoles with Species A by Association with Infected Tadpoles of Rana pipiens.*—Evidence was presented above that tadpoles of *Rana pipiens* can be infected with euglenoids of Species A by feeding them food containing active trophozoites, but this probably is not the method of infection in nature. Inasmuch as all tadpoles in certain ponds were found to be infected and none or a very few in other ponds, infection by association was suggested. To test this method the following experiment was carried out. A large number of toad tadpoles were collected on July 10. Five of these were examined on July 23 and found to be uninfected. Seven of the remaining tadpoles were then placed in a finger bowl with 7 tadpoles of *Rana pipiens* taken from a lot that were all infected. They were kept together for five days. Three of the toad tadpoles were still alive, three had recently died, and one had died previously and been removed. Each of the three living toad tadpoles, on examination, was found to contain large numbers of Species A in the rectum and a few in the intestine. No specimens were found in one of the dead tadpoles but a few were present in the other two dead tadpoles. At this time ten more toad tadpoles from the control lot were examined; eight of these were uninfected and one specimen of Species A was found after careful search in the rectum of each of the other two. These results prove that uninfected tadpoles may become infected by associating closely with infected tadpoles. The obvious way in which this is brought about is the escape of specimens from the rectum of infected tadpoles into the water and their ingestion by the uninfected tadpoles. This probably occurs in nature only when the tadpoles are closely associated. Toad tadpoles are very gregarious and might easily infect one another even in a large pond. Tadpoles of other species are much less accustomed to congregate in numbers and hence would not transfer their infection unless confined to a small body of water.

VIII. *Species A in Tadpoles of the Green Frog, Rana clamitans.* —Euglenoids of Species "A" were encountered in very small numbers in tadpoles of the green frog. For example, several specimens were found in the rectum of each of four tadpoles that were collected on June 14 and kept in the laboratory without food until June 26. That these euglenoids may be taken in with the food of the tadpole and localized in the rectum is evident from the following experiment. One tadpole of the green frog collected on June 14 was starved until June 28 and then fed the rectal contents of three toad tadpoles containing euglenoids. Two days later a considerable number (two per field) of Species A were found in the recutm of this specimen—a much greater number than had been found in any of the several hundred other green frog tadpoles previously examined. Furthermore most of these specimens were pale in color like those in the toad tadpoles at this time.

IX. *Specificity of Euglenoids of Species A.*—It seems probable from the observations and experiments recorded above that euglenoids of Species A are "normal" inhabitants of the recutm and intestine of tadpoles of the frogs and toads that inhabit freshwater ponds. Those living in different hosts may be specifically distinct but no evidence of this was obtained. Attempts to infect adult salamanders proved negative. Ten infected toad tadpoles were fed to a specimen of *Diemyctylus viridescens* on July 1. Four days later no traces of euglenoids could be found in the digestive tract of this specimen, indicating that this salamander cannot be infected with its food and that these euglenoids are probably unable to live in adults of this species. Another specimen of this species of salamander was placed in a dish containing millions of free-living euglenoids. Four days later, the intestine of this animal contained green chlorophyll masses, but no recognizable euglenoids.

X. *Euglenoids of Species B.*—A second type of euglenoid was noted in the rectum and intestine of tadpoles of *Rana pipiens* and *R. clamitans*. Euglenoids of this species, which may be referred to here as Species B, probably were present in all of many *Rana pipiens* tadpoles collected when about 9 weeks old. They were recorded incidentally in 32 of them, although very little attention was devoted to them at the time. They were noted also in five large tadpoles of the green frog but were probably also of general occurrence in this species. Species B is a cylindrical, elongate species with a blunt anterior end and a posterior end terminated either by a short subacute tip or by a longer process which is slender, pointed at the tip and slightly curved. No free swimming forms were seen but most of the specimens were undergoing slow metabolic movements. Specimens ranged from 99 μ to 128 μ in length and from 9 μ to 16 μ in breadth, with an average length of 110 μ and average breadth of 12 μ. A single short flagellum that moved too slowly to disturb the particles in the surrounding medium was observed in several specimens. Numerous disciform green chromatophores and a large reservoir and red stigma are present. The periplast is spirally striated and strongly punctated in some specimens. In many respects it resembles *Euglena spirogyra* Ehren. Fig.4 is a camera lucida sketch of a living specimen magnified 1600 diameters.

Euglenoids of Species B were never numerous in any tadpole. They were more often observed in the intestine than in the rectum. Their ability to live in these environments and their frequent occurrence indicate that they are normal inhabitants of the digestive tract of tadpoles. An attempt was made to increase their numbers by feeding the rectum and intestine of infected tadpoles to other infected tadpoles. Thus the digestive tracts of 22 tadpoles were fed to four tadpoles of *Rana pipiens* on July 4. Not all of this food was eaten and after 24 hours living active specimens of both Species A and Species B were observed within the walls of the intestines and recta offered as food. Specimens were still alive and active after 48 hours. One tadpole was killed and examined after an interval of three days (July 7) and the remaining three after six days (July 10) but no increase in the numbers of Species B could be ascertained with certainty. I believe there was an increase but the actual numbers were too small to determine the point definitely.

XI. *Species of the Genus Phacus.*—In a few of the tadpoles of

Rana pipiens a species of *Phacus* was noted that resembled in size and shape, *Phacus pleuronectes*. They were about 42 μ long and 32 μ broad, oval in shape with an uncinate posterior spike about 6.5 μ long, a longitudinal groove extending anteriorly from the posterior end, a longitudinally striated periplast, and a large number of green chromatophores, as indicated in Fig. 5. These organisms remained alive and active for 48 hours in intestinal contents that were sealed under a cover glass with vaseline.

XII. *The Effects of the Presence of Euglenoids on Other Intestinal Protozoa.*—No apparent effects on the number and distribution of other intestinal protozoa were noted due to the presence of Euglenoidina. There was, however, a change in the color of many of the opalinas. As a rule *Opalina* is almost transparent when studied with a compound microscope but whenever euglenoids were abundant many of them took on a greenish yellow tinge. This was true in the tadpoles of both *Rana pipiens* and the toad and was very noticeable in the tadpoles that were fed on free living euglenoids. Evidently nutrition in *Opalina* in the presence of chlorophyll bearing bodies is a process whereby some of this coloring matter is taken in thus causing a greenish tinge.

XIII. *The Parasitic Habit among the Euglenoidina.*—Only a few of the Euglenoidina have been described as parasitic in habit. Prof. L. B. Walton mentions two species of the genus *Astasia* in his monograph on the Euglenoidina (Walton, 1915) and has called my attention (in litt.) to other members of the order described by Alexeieff (1912) and Nieschultz (1922). *Astasia captiva* is endoparasitic in *Catenula lemnae*, and *A. mobilis* in the egg sacs and digestive tract of *Cyclops*—a condition that has led to the suggestion by Alexeieff that parasitic Sporozoa may have originated in this way. Nieschultz describes a species of *Astasia* from the digestive tract of a fresh-water nematode, *Nilopus gracilis*, but does not give it a specific name. Haswell (1907) reported the occurrence of a euglenoid inside the cells of a rhabdocœle Turbellarian. Alexeieff reports the discovery of small, living, active euglenoids, *Euglena* sp. and *Phacus longicauda*, in frog tadpoles and states that Brumpt also showed him specimens containing hundreds of *Euglenæ*. The green color and stigma of these were as bright as in free-living specimens. This, according to Alexeieff, is a case of accidental parasitism which is a stage in the evolution of parasitism among the Euglenoidina. He accounts for the presence of these euglenoids by the mode of nutrition of the tadpoles, which engulf large quantities of debris of all sorts resulting in a thick, compact mass in the intestine. Organisms in the midst of such a mass might easily escape the action of the digestive juices, especially since these are greatly diluted, and as a result become gradually acclimated and finally facultative parasites.

XIV. *Summary and Conclusions.*—(1) Three species of Euglenoidina are described from the intestine and rectum of frog and toad tadpoles; all three species possess green chromatophores and bright red stigmas.

(2) A comparative study of two sets of tadpoles of *Rana pipiens* from adjoining ponds, one set much retarded in growth and heavily infected with Species A and the other of normal growth but lightly infected or not at all, indicates that the dwarfing of the former may have been due to the presence of euglenoids.

(3) Tadpoles of *Rana pipiens* infected with Species A were kept in the laboratory without food for 25 days and specimens examined at intervals. The infection persisted throughout this period without any marked decrease in the brightness of the green color of the euglenoids. Only one case of division was noted, and no cysts were found, the euglenoids remaining as trophozoites, free swimming and actively metabolic, throughout the period. The retention of the green color may have been due to the transmission of light through the almost transparent abdominal and intestinal wall. The rectum is the usual habitat of this species but the intestine is often invaded especially the first 10 mm. just anterior to the rectum. They are not mixed with the intestinal and rectal contents but move about between this mass and the containing walls. Infection was found to persist in tadpoles collected from time to time from the pond.

(4) Tadpoles of *Rana pipiens* containing very few specimens of Species A were fed on the intestines and recta of highly infected tadpoles of the same species. A great increase in the number of Species A in the experimentally fed tadpoles proves that infection with this species can be brought about by the ingestion of active trophozoites with the food. This, however,

is probably not the usual method of infection in nature, since a resistant over-wintering form of Species A probably exists by means of which the new broods of tadpoles are infected in the spring.

(5) Attempts to cultivate Species A outside of the tadpole failed and it seems probable that trophozoites are incapable of living and reproducing themselves in water outside of the host. Specimens were kept for several days in intestines and recta that had been dissected out and placed in water and also in rectal contents sealed under a cover glass. The latter proved to be sensitive to light congregating on the side of the slide toward a north window and moving from one side of the slide to the other, a distance of 16 mm., in about 20 minutes, when placed opposite this window.

(6) An attempt was made to infect tadpoles of *Rana pipiens* with three species of free-living euglenoids. The euglenoids were all ingested but none became colonized in the digestive tract. One of these euglenoids was taken from the bladders of *Utricularia* in which they were able to maintain themselves in spite of the secretions present there. The species that inhabit the intestine and rectum of the tadpoles therefore possess a resistance to digestive juices not present in free-living forms.

(7) All of a large group of tadpoles of the toad, *Bufo lentiginosus americanus*, were found to be infected with Species A, but another group of tadpoles of this species from another pond were not infected. Specimens of infected tadpoles were kept in the laboratory without food for 31 days and examined at intervals. The euglenoids persisted throughout this period. No apparent increase in numbers was noted and no division stages nor cysts were observed. There was no decrease in size. The organisms were free-swimming and actively metabolic. The green chromatophores, however, gradually became paler in color until the specimens were almost transparent. This loss of the green color was probably due to the failure of sufficient light to penetrate the deeply pigmented abdominal wall of the toad tadpole.

(8) Euglenoids of Species A were also found in the intestine and rectum of tadpoles of the green frog, *Rana clamitans*. They were not as numerous as in tadpoles of *Rana pipiens* or of the toad. One tadpole that was fed on the intestines and recta of three infected toad tadpoles became more highly infected, thus proving

that active trophozoites from the latter can be transferred to tadpoles of the green frog with their food.

(9) It is evident that euglenoids of Species A are regular inhabitants of the intestine and rectum of three species of tadpoles, *Rana pipiens*, *R. clamitans*, and *Bufo lentiginosus americanus*, and that they can be transferred from tadpoles of one species to those of another with the food. No specific differences were noted in specimens from different species of tadpoles.

(10) Euglenoids of Species B were present in tadpoles of *Rana pipiens* and *R. clamitans*, but were never very numerous. The intestine seemed to be more highly infected than the rectum. The specimens observed were as green as free-living species and contained brightly colored stigmas. Most of them appeared to be without flagella and either remained stationary except for metabolic movements or squirmed slowly from place to place. Attempts to increase the number present in one tadpole by feeding it infected intestines and recta of other tadpoles were not definitely successful.

(11) A species of *Phacus* resembling *P. pleuronectes* was observed in a few tadpoles of *Rana pipiens*.

(12) The presence of euglenoids had no apparent effect on other protozoan inhabitants of the digestive tract except in the case of certain opalinids which became yellowish green in color.

The advantages of the group of organisms dealt with in this paper as material for a study of the evolution of parasitism is obvious and the writer expects to continue work on the group with this object in view.

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BIOLOGICAL BULLETIN

THE AXIAL GRADIENTS IN HYDROZOA. V. EXPERIMENTAL AXIAL TRANSFORMATIONS IN HYDROIDS.

C. M. CHILD.[1]

The hydroid stolon usually appears in nature as a basal outgrowth readily distinguishable from the stem by its habit of growing in contact with surfaces rather than free in the water. Contact has often been regarded as a factor in determining its formation, but it has been observed by many investigators that in the reconstitution of isolated pieces of hydroid stems stolons may develop from that end of the piece which was originally apical, as well as from the basal end, even when these ends are not in contact with solid surfaces. In some cases also it has been observed that isolated pieces of certain species develop only stolons which may later give rise to hydranths or may continue to grow as stolons. Peebles (1900) states that this frequently occurs in pieces of *Hydractinea* and *Podocoryne* when they are left in dishes undisturbed without change of water. Loeb ('92) maintained that stolon formation in *Antennularia antennina* is determined by gravity, but Morgan ('01) and Stevens ('02, '10), while not denying the correctness of Loeb's conclusions, demonstrated beyond a doubt that other factors than gravity may be concerned. They were not able, however, to reach definite conclusions concerning the nature of these factors. Lund ('21) has shown that when isolated stem pieces of certain hydroid species are exposed to the electric current, stolons tend to arise at the end toward the cathode, hydranths at the end toward the anode. Various other data might be cited, but it seems fair to say that no one thus far has been able to discover a general physiological basis for the development of stolons in hydroids. The original polarity, gravity, contact, the electric current, light, have all been mentioned as factors concerned in determining the polarity of hydroids, but no one of these has been shown to be of fundamental or general importance as regards stolon formation.

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[1] From the Hull Zoölogical Laboratory, University of Chicago.

The present paper is concerned with observations and experimental data which throw some further light upon this problem. These data were accumulated during four summers, 1917–1920, spent at the Puget Sound Biological Station, and I take this opportunity of expressing again my obligation to the Director for the facilities afforded.

Material and Methods.

Three hydroid species, *Bougainvillea mertensi*, *Obelia borealis* and *Gonothyræa clarkii*[1] were chiefly used, but a few experiments were performed with other species.

In the course of other work with hydroids it was observed that often in *Bougainvillea*, and occasionally in other forms, freshly collected stocks showed stolons in place of hydranths. Commonly these stolons were found in the basal one fourth to one half of the stock and on the more basal secondary branches of the primary branches of this region. In some stocks this basal portion is a tangle of stolons with few or no hydranths, the stolons arising in part in the positions of hydranth buds and in part as apparently "adventitious" outgrowths.

A little later it was observed, particularly in *Bougainvillea*, that stocks which were entirely without these stolons when first collected often developed them after a few days in standing water in the laboratory. This suggested the possibility that such transformation of hydranth buds into stolons might be the result of depression or inhibition and experimentation with low concentrations of various inhibiting agents and conditions, such as crowding in standing water, keeping in closed dishes, etc., was begun.

In these experiments the following chemical agents and concentrations[2] were used: KNC *m*/10000, *m*/25000, *m*/50000, ethyl urethane, *m*/200, *m*/500; $MgSO_4$, *m*/400, *m*/1000; LiCl,

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[1] I am indebted to Dr. C. C. Nutting for the identification of the *Obelia* and to Dr. Trevor Kinkaid for the identification of the *Bougainvillea* and *Gonothyræa*.

[2] Concentrations as given represent merely equivalents in sea water.

m/50; HCl, *m*/1500, *m*/5000; neutral red. With some concentration of each of these agents positive results were obtained, and crowding, keeping in closed dishes, and infrequent change of water also gave positive results.

In the experiments complexes, stocks or "colonies" lacking only the holdfast, or large complexes of stems and branches with a single cut surface at the basal end were used. These were placed in finger bowls holding about 400 c.c. of the experimental solution made up with well aërated sea water. When volatile agents were used the bowls were completely filled and covered with glass plates excluding all air, or all except a small bubble. Solutions were renewed daily or every two days, except in experiments to determine the effects of less frequent change. And finally, in some experiments the same concentration was continued throughout, in others the original concentration was replaced by a lower one, or the animals were returned to water after a day or two.

The figures are semi-diagrammatic but are all drawn from living specimens. Old stems, branches or thecæ which are empty because of disintegration or resorption of hydranths or retraction of cœnosarc are drawn in broken lines (Figs. 13–16, 18). Figs. 1–9, 11, 13 representing the development of stolons in earlier stages of experiment are drawn in outline without indication of cœnosarc, because all parts of stems and stolons contain it. In the other figures the cœnosarc is indicated by shading in order to show the later development and separation of stolons from the stock.

The chief purpose of the paper is the presentation of experimental data which show that transformation of stems and even of apical regions into stolons may occur under slightly inhibiting or depressing conditions. Questions of the range of effective concentrations, of regional, individual, specific and experimentally induced differences in susceptibility, and of the rate and degree of transformation are considered only incidentally or not at all.

Transformation in *Bougainvillea*.

Transformation of apical ends of branches into stolons often occurs in nature in *Bougainvillea*. In freshly collected stocks stolons are often found in place of some or all hydranths and buds of the more basal regions, usually not more than the basal third or half, or as adventitious outgrowths from the branches of these regions. What factors are concerned in such cases is of course uncertain, but it may be pointed out that in these basal regions, which are often over-grown with plants and protozoa, less favorable conditions for respiratory exchange—retarded water movement, accumulation of CO_2, lack of oxygen—may directly inhibit and transform hydranth buds into stolons. On the other hand, if the whole stock is exposed to inhibitory conditions, the more basal regions, because of their lower rate of metabolism (Child, '19, '21; Hyman, '20), are in general less able to acclimate to such conditions than the more apical regions and may, therefore, undergo transformation, while other levels of the stock do not. Stocks which show no stolons at any level above the basal end when collected usually develop stolons over more or less of the stock after a day or two in standing water.

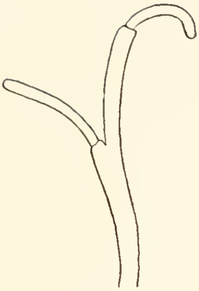
That such development of stolons from the more basal regions of stocks in water is not due to the presence or accumulation of "stolon-forming substances" in these regions or to any other specific factor is shown by the fact that this transformation can be induced in any or all regions of the stock, according to experimental conditions. The following experiments serve as examples:

1. Pieces of stocks in ethyl urethane, *m*/200. After forty-eight hours all hydranths disintegrated and numerous stolons developed, chiefly apical (Fig. 1) or subapical (Fig. 2).

2. Pieces in ethyl urethane for twenty-four hours, then returned to well aërated sea water. In ethyl urethane all original hydranths disintegrated and stolon development began. After twenty-four hours in water many subapical stolons were present, but new hydranths were developing and some stolons were transforming into hydranths and stems.

3. In ethyl urethane *m*/500 the original hydranths disintegrated and apical and subapical stolons developed within twenty-four hours, but after forty-eight hours many apical ends and in some cases the first subapical bud developed new hydranths and the stolons were inhibited (Fig. 3).

4. Pieces in ethyl urethane *m*/500 twenty-four hours, then returned to water, gave much the same results, except that in some cases the tips of the apical stolons themselves transformed



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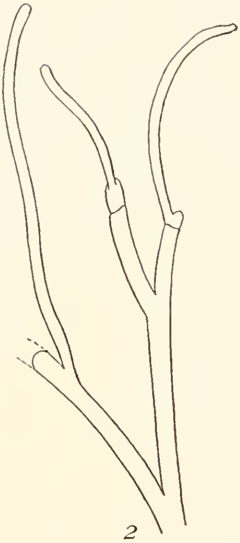

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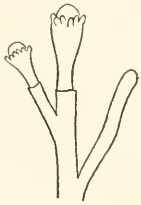

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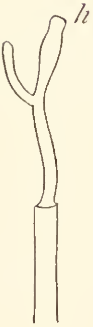

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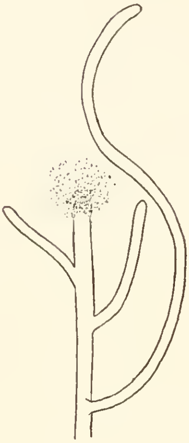

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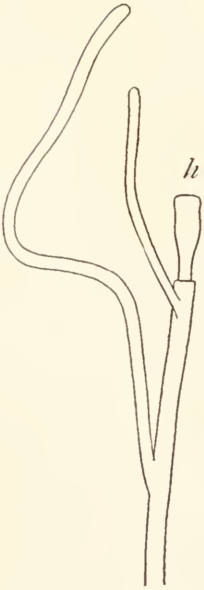

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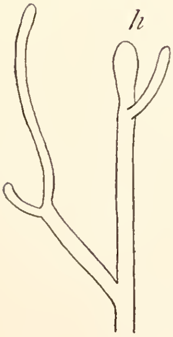

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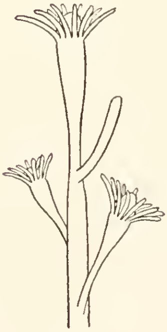

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into hydranths after return to water (Fig. 4; *h*, hydranth bud).

5. In $MgSO_4$ *m*/400 almost all hydranths were disintegrated and subapical stolons grew rapidly within forty-eight hours (Fig. 5).

6. In $MgSO_4$ *m*/1000 disintegration of hydranths occurred and stolons developed much as in Expt. 5, but after forty-eight hours many new apical hydranths developed (Fig. 6; *h*, hydranth bud).

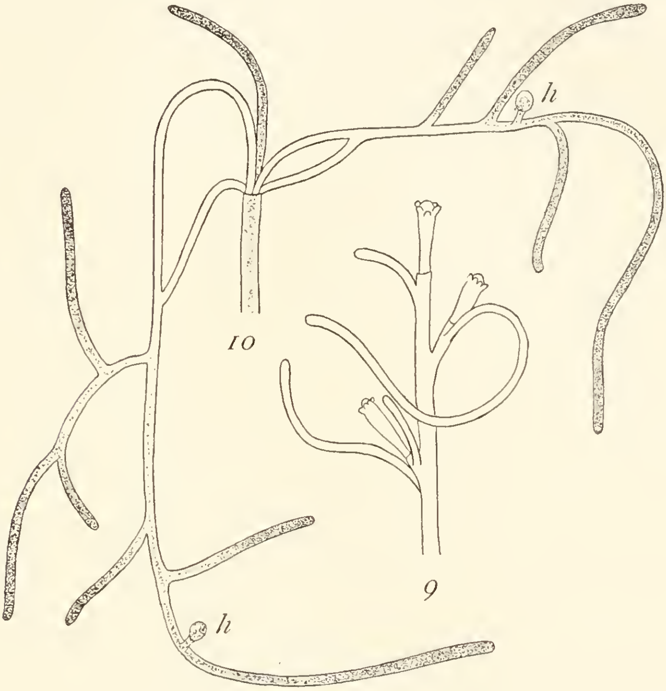
7. In sea water controls of Expts. 1–6 after forty-eight hours the original hydranths were more or less affected: tentacles were undergoing reduction or were completely gone and some hydranth bodies were disintegrated or disintegrating, or, in the case of the younger hydranths, resorbed. Subapical, or even apical stolons appeared on those axes in which the terminal hydranths were reduced or disintegrated (Fig. 7) and rarely short stolons appeared on stems with persistent hydranths (Fig. 8). On some stems new hydranths were developing Fig. 7, *h*).

8. In KNC *m*/10000 hydranths became motionless within a few hours and in the course of three or four days disintegrated, but in the course of six days no stolons developed. Solution was then changed to *m*/20000 and left exposed to air to permit slow decrease in concentration. A few short, subapical stolons developed during the next eight days, but no development of new hydranths occurred and medusa buds underwent resorption. Evidently development and growth were almost completely inhibited by this concentration.

9. In KNC *m*/25000 older hydranths disintegrated and younger underwent resorption. Stolons developed in subapical and lower regions. After six days solution was changed to *m*/50000 and left exposed to air. During next eight days an extensive stolon system developed from nearly all apical ends of all stems and branches and also from basal cut ends of pieces. No hydranths were present but a few hydranth buds appeared on the stolons. Results essentially like those of following experiment.

10. In KNC *m*/50000 the original hydranths disintegrated and stolons appeared and after six days small, partially inhibited new hydranths were present (Fig. 9). Solution was then changed to *m*/100000 in open dishes to permit gradual decrease in concentration. During the next eight days the new hydranths were

resorbed and extensive stolon systems developed from practically all tips (Fig. 10). These stolons bear a few erect stems with hydranth buds (Fig. 10; *h*, *h*), but these did not develop further.

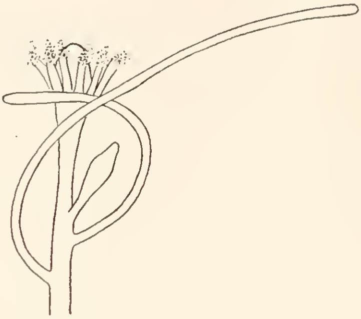


11. In HCl $m/5000 \pm$ pH 7.4, after forty-eight hours, most terminal hydranths of upper branches disintegrated and many subapical stolons present. Many hydranths at lower levels intact; no stolons. In controls in sea water, changed daily, terminal hydranths mostly disintegrated but new hydranths developing and very few stolons present.

12. In HCl $m/1500 \pm$ pH 6.9 hydranths disintegrate in one to two days, terminal hydranths usually first. After three days numerous stolons appeared in basal halves of pieces, but all development was inhibited in the apical halves. In sea water controls after three days the original hydranths were gone, many new hydranths were developing and few stolons appeared.

13. In pieces kept in standing water in closed vessels without air the changes during the first forty-eight hours are essentially

similar to those occurring in open dishes. More or less reduction and disintegration of hydranths and development of stolons occurs. Figure 11 shows the apical region of a stem in which the original terminal hydranth has reduced tentacles with disintegrating tips and the subapical hydranth bud has advanced but little during the forty-eight hours since collection. Below the bud two stolons have developed. In some other pieces in the same dish most or all hydranths appear intact, and no stolons or very few, chiefly in basal regions, are present. In still others apical hydranths are already completely disintegrated and stolons are growing rapidly.



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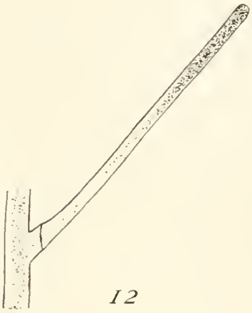
In all cases in standing water the original hydranths disappear sooner or later and stolons develop, but a second and even a third generation of hydranths may arise. After a week or ten days, however, hydranths are usually entirely absent and all tips have either given rise to stolons or the cœnosarc is retracted. In short, the effect of standing water is essentiallly the same as that of the inhibiting agents, but less rapid. It is probably due in large part to accumulation of CO_2. There is always a decrease in pH in the dishes containing the pieces, often to 7.5 or 7.4 in the course of a week or less.

Transformation in *Gonothyrœa*

Gonothyrœa is much less susceptible to inhibiting agents and to standing water than *Bougainvillea.* The hydranths survive for several days or even a week under conditions which kill the hydranths of Bougainvillea in a day or two.

Some of the earlier experiments gave negative results as regards stolon formation, probably because the inhibiting conditions were not sufficient in degree or not continued long enough. Apparently however, the transformation occurs under less extreme conditions in stocks which have begun to produce gonozooids



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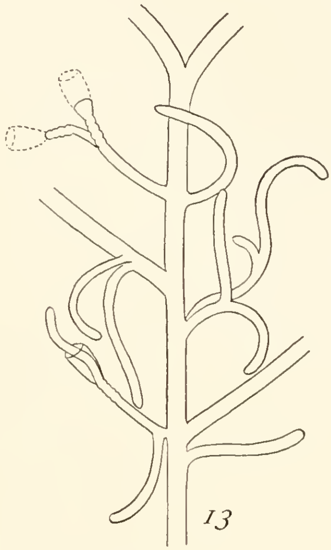

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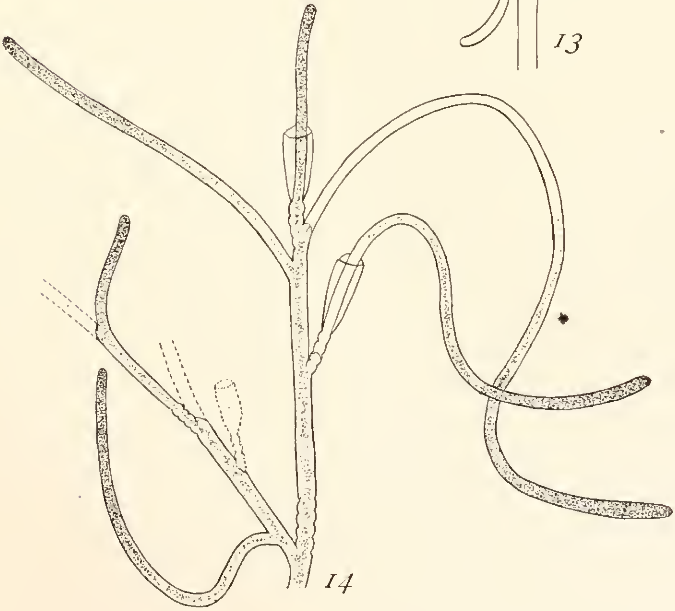

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than in those which have none, but my data indicate that transformation will occur to some extent in any stock with sufficiently high concentrations of the agent used and sufficient time. Lack of material limited experiment with this species. A few examples are given.

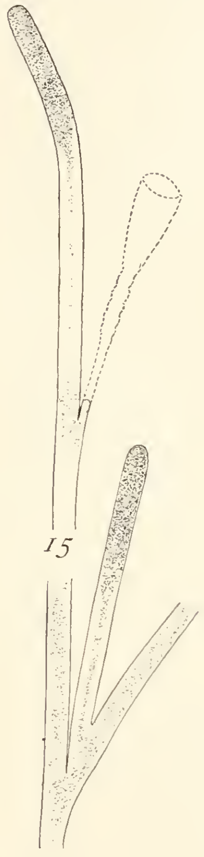
Pieces in HCl *m*/2500 ± (pH 7.3) still possess some hydranths after four days. A few short outgrowths, apparently intermediate between stems and stolons have developed but no typical stolons. These pieces were then changed to HCl *m*/1500 ± pH 6.8 and after two days more showed numerous stolons in the more basal regions and outgrowths apparently intermediate between stolons and stems in the apical regions. These intermediate outgrowths are straight, support themselves free in the water and do not adhere to surfaces as do the stolons, but they show no annulation and they develop quite independently of hydranth buds (Fig. 12).

Fig. 13 shows the development of stolons in the basal region of a stock after a week in ethyl urethane *m*/200. In ethyl urethane *m*/500 transformation did not occur within a week. Figure 14 shows stolon development in the apical region of a stock after ten days in standing water. This stock bore numerous gonozooids in the basal regions.

TRANSFORMATION IN *Obelia*.

In *Obelia borealis* transformation was observed in HCl *m*/1500, LiCl *m*/50, chloretone *m*/2000, neutral red and also in standing water, but in water the stolons are less numerous than in the various solutions and after a few days new hydranths develop. In neutral red all parts stain opaque black, the hydranths disintegrate or are resorbed and stolons which are opaque black like other parts develop in large numbers. In this species stolons may be either terminal (Figs. 15, 16) or in the axils of branches (Fig. 17). Usually when they attain a certain length they separate from the parent stock, fall to the bottom and the tip continues to grow at the expense of lower levels until reduction to minute size and exhaustion occurs, or, if inhibiting conditions are removed, they may give rise to hydranth and stem.

Obelia geniculata behaves essentially like *O. borealis* as regards transformation and the stolons are of the same type in the two species. Another unidentified campanularian showed extensive stolon formation after two days in standing water, many of the stolons being apical. Fig. 18 shows a case of apical transformation in this species.



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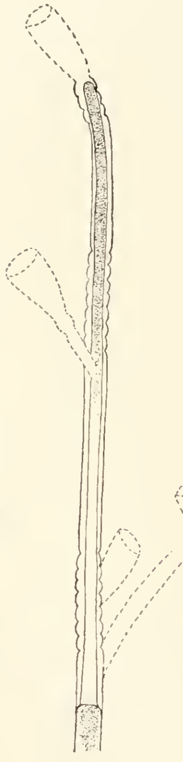

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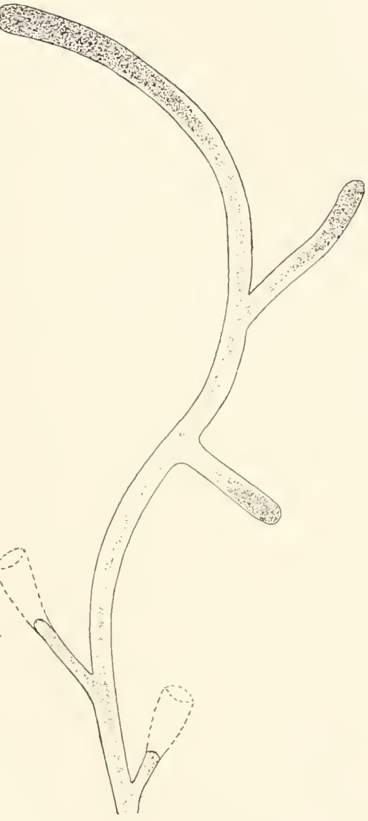

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Differences between Stolons and Stems.

There is usually no difficulty in distinguishing a stolon from a stem. The stem supports itself and is relatively rigid, the stolon is much less rigid and when it grows free in the water tends to hang downward as its length increases, until it comes into contact with a solid surface. Once in contact with a surface, the perisarc of the stolon adheres to it and its further growth is along the surface, but the stem does not attach itself to surfaces. It is not true, however, that contact is an essential factor in the origin of stolons, but my observations suggest that in general stolons in contact grow to greater lengths and perhaps more rapidly than those free in the water. In the figures all the longer stolons are in contact with the glass of the container over most of their length (Figs. 2, 5, 10, 14, 18). The adventitious stolons in Figure 13 and the short stolons of Figures 1–5, 7, 8, etc., are not yet in contact with surfaces.

The stem grows only as part of a hydranth-stem complex or gradient, while the stolon may continue to elongate indefinitely without developing hydranths or hydranth buds. The direction of growth of stolons is apparently indefinite, or at least is readily altered, but the stem is usually straight. And finally, in those species in which annulations of the perisarc appear on the stems, the stolons are not annulated.

The "intermediate" outgrowths observed in *Gonothyræa* in HCl (p. 190 and Fig. 12) resemble stems in their rigidity and definite direction of growth, but, except in the development of the first hydroid from the planula, stems do not grow out in this manner without at least a hydranth bud at the tip. If the difference between stolon and stem is primarily one of "steepness" or other difference of the gradient, such intermediate outgrowths are possible and there is no good reason for doubting that the outgrowths observed are really intermediate.

The Process of Transformation.

The first step in the process is the inhibition or depression of the hydranths. The changes in the hydranths differ according to species and degree of depression, and range from decrease or cessation of motor activity for a day or two with subsequent recovery to complete disintegration or resorption. In *Bougainvillea* all the original hydranths usually disappear, even in standing water, within twenty-four to thirty-six hours and new hydranths may begin to develop within forty-eight hours. In *Gonothyræa*, on the other hand, the change from natural conditions to standing water has little effect on the hydranths; they remain intact for a week or more in most cases, but finally disintegrate or are resorbed as starvation advances.

The older, fully developed hydranths apparently always die and disintegrate, at least in large part, in all the species observed, though it is possible that some portions may be resorbed, but younger developing hydranths or buds may be completely resorbed without visible loss of tissue. In *Bougainvillea* even the younger medusa buds underwent complete resorption under the more extreme inhibiting conditions, *e.g.*, KNC *m*/10000 six days, then gradual decrease in concentration. After two weeks the youngest medusa buds were completely resorbed, only the empty perisarc marking their position, somewhat older buds were partly resorbed and the most advanced buds had undergone disintegration.

The question of the nature of the process of resorption of hydranths was discussed some twenty years ago by Loeb ('00) and Thacher ('03), Loeb postulating a liquefying enzyme and Thacher interpreting the process as a degeneration. To all appearances the process is to some extent a real dedifferentiation. There is no visible loss of tissue and from what we now know of metabolic relations in the hydroids a simple interpretation of resorption and retraction of parts seems possible. The growing hydranths and medusa buds are regions of higher metabolic rate than the stems and are therefore able to appropriate the larger share of nutrition, but at the same time they are more susceptible to inhibiting or depressing conditions than stems (Child, '19, '21). When subjected to these conditions their metabolic rate decreases to a much greater degree than that of stems. Under such conditions they may not only be unable to maintain themselves, but their tissues, instead of taking nutriment from other parts, may become, whether through autolysis or other factors, a source of food for other parts. Consequently they may undergo decrease in size until nutritive equilibrium is established, or until the region with lower metabolic rate is more or less completely resorbed. Whether individual cells actually die in such a process or merely undergo dedifferentiation is difficult to determine, but in the light of what we know of the possibilities of dedifferentiation in these animals, it seems probable that cell death does not necessarily occur.

In starving hydroid stocks in the laboratory the retraction of cœnosarc in some regions and its outgrowth in others has often been noted. Tests of susceptibility and of permanganate reduction in such stocks indicate that the regions which are growing at any given time are regions of high rate of oxidation, while those which are undergoing retraction or resorption are regions of low rate. It seems probable then that in general the retraction of one part and the growth of another, particularly in starving stocks are associated with such differences in rate, the region of high rate maintaining itself and even growing at the expense of less active regions.

The normal relations and proportions of parts in nature must depend in large measure upon certain relations of rate of fundamental metabolism. When these metabolic relations are altered the form or proportions must change, and in simple, highly plastic forms such as hydroids, such changes may involve the complete resorption or atrophy of previously existing parts and the dominance and development of parts previously subordinate.

The stolon represents a physiological axis, a gradient (Child, '19, '21), but the data of susceptibility, $KMnO_4$ reduction and vital staining for some ten hydroid species examined indicate clearly that the stolon gradient is much less "steep" than the hydranth-stem gradient and that whenever, and wherever the gradient in a stolon becomes steep enough, transformation into a hydranth-stem complex occurs. In other words, the difference in rate between the hydranth bud or hydranth and the stem is greater than that between the stolon tip and the stem or the lower levels of the stolon.

According to this viewpoint, the stolon usually appears in nature as a basal structure, not because of the presence there of any "stolon-forming substances" but first, because this is the region of lowest metabolic or oxidative rate in the stock, and second, because new buds arising in this region are more or less inhibited by the dominance of the more active regions above. Probably the bioelectric currents resulting from the differences in electric potential between basal and higher levels are important factors in such inhibition. But whatever the factors involved, the partially inhibited axis develops in the form of a stolon. As I showed for *Tubularia* (Child, '15, pp. 91–2, 130–37), when the distance of the stolon tip from the hydranth becomes great enough, the stolon tip becomes physiologically isolated from the inhibiting action of the more active levels and transforms at once into hydranth and stem.

The experiments show, however, that stolon formation is not necessarily limited to basal regions of the stock, but may occur anywhere, even at apical ends, under inhibiting external conditions. In consequence of the differential susceptibility of different levels of the axis, the effect of such conditions is to decrease the steepness of the gradient. This change induces disintegration or resorption of hydranths, and new buds, instead of developing into hydranths, give rise to stolons. Moreover, a greater or less degree of physiological isolation of stem regions results from the disappearance of the dominant hydranths and in some species, as in many plants "adventitious" buds, *i.e.*, buds not localized in conformity to the usual order, arise (Figs. 11, 13). But under the inhibiting conditions the buds develop as stolons, not as hydranths. Such adventitious stolons have been seen most frequently in the basal halves or thirds of *Bougainvillea* and *Gonothyrœa* stocks, but apparently may occur anywhere. And finally, the inhibiting conditions alter the steeper hydranth-stem gradients of terminal regions into the less steep stolon gradients and stolons therefore appear in place of hydranths.

Often more or less acclimation to the inhibiting conditions occurs in the course of a few days, and new hydranths begin to develop either from terminal regions which have not formed stolons (Fig. 7), from new buds, or by the transformation of stolon tips (Fig. 4). Such hydranth development retards or completely inhibits further growth of subterminal stolons near the hydranth, but the growth of terminal stolons may continue indefinitely (Figs. 10, 14, 15, 18) unless conditions are so altered as to induce their transformation into hydranth-stem gradients. In the case shown in Fig. 10, for example, each of the two chief stolon outgrowths gave rise at one point in its growth to a hydranth bud (*h*, *h*), but these two buds were unable to develop further than the stage shown in the figures and later underwent resorption.

SEPARATION OF STOLONS FROM THE STOCK.

In all species investigated continued growth of the stolon leads sooner or later to loss of continuity between its cœnosarc and that of the parent stock. In *Bougainvillea* (Fig. 10) and *Gonothyraea* (Fig. 14) such separation of stolon and stock usually occurs gradually and only after the stolon attains considerable length, but in *Obelia* it usually occurs at a relatively early stage and the region of separation is more sharply localized (Figs. 15, 17). Such differences, however, are not entirely constant for the species, for *Bougainvillea* stolons sometimes show a definite level of separation and *Obelia* stolons sometimes do not.

After separation of the cœnosarc the stolon may remain connected with the stock by the perisarc. In the case of stolons hanging free in the water the empty perisarc usually breaks and the stolon falls to the bottom, attaches itself and continues to grow, the tip growing at the expense of more basal levels until exhaustion occurs, or until conditions are altered so that the tip can transform into a hydranth and stem. Such free stolons may cover many centimeters of distance, leaving behind them a tube of empty perisarc as they go, and decreasing in length as their substance is gradually used as nutrition. In the laboratory this growth may continue for three weeks or even more, according to temperature, and while transformation into hydranths often occurs in the early stages, it apparently does not take place, even in favorable environment, in the later stages, but the stolon continues to "creep" over the bottom until reduced to a minute amount of cellular material. And even when growth ceases the small masses of tissue in the perisarc remain alive for some time longer.

The separation and continued growth of these stolons receives a simple physiological interpretation in terms of the axial gradient. If the stolon is such a gradient, the levels of relatively high rate are able to live to some extent at the expense of lower levels. Under laboratory conditions, without intake of food, the growth of the stolon tip is possible only at the expense of other parts. In the early stages the stolon tip, as a region of higher metabolic rate than the old stem cœnosarc, is able to take material from the latter, but as the stolon elongates the growth of the tip occurs more and more exclusively at the expense of the lower stolon levels, because the stolon gradient, and consequently the nutritive concentration gradient, is limited in length and when the length of the stolon exceeds this limit, it can no longer draw on the stock for nutrition.

From this stage on, the lower levels of the stolon gradient gradually lose material to the higher levels and finally the cœnosarc of these levels disappears completely and separation of the stolon occurs. In Fig. 10 the two chief stolons have already separated from the original stock, and some of their longer branches are approaching separation from each other. In Fig. 14 also the two largest stolons are separated and a third is approaching separation. In Fig. 15 the terminal stolon of *Obelia* is almost separated, in Fig. 16 separation is complete, except as regards perisarc, and in Fig. 17 an earlier stage is shown.

After separation growth goes on as long as the regions of higher rate are able to take material from those of lower rate. In such stolons, even after a week or two of growth, the tip appears well fed and the cell layers are thick while toward the base the layers become progressively thinner and the cells more shrunken.

It is not yet known whether the rate of oxidation increases in advanced starvation in hydroids as it does in *Planaria* and various other animals, but apparently either this occurs at the lower levels more rapidly then at the upper levels of the separated stolon, or else the rate of oxidation in the upper levels decreases as the supply of nutritive material decreases. Either change leads gradually to the obliteration of the gradient, and as the cells become more and more alike in condition, growth becomes slower and slower and finally ceases.

According to this interpretation then the continued growth of such separated stolons in the absence of food from without is a simple physiological consequence of the fact that they represent physiological gradients and likewise the difference in appearance of the cœnosarc from the well filled tip to the shrunken, almost transparent base is another expression of the gradient. It may be suggested further that such stolons fail to develop hydranths in the more advanced stages of starvation because the gradient cannot attain the steepness necessary for hydranth formation. So far as I know, no other adequate physiological interpretation of these various facts has been advanced. The separation and continued growth of stolons may be an adaptation for purposes of reproduction under unfavorable conditions, but even if this is the case, the necessity for physiological interpretation still exists.

CONCLUSION AND SUMMARY.

It is evident that the formation of stolons in the hydroid species investigated is not dependent on region of stock, gravity, or contact, but rather on a certain degree of inhibition or depression, which may be determined either by relations to other parts of the stock or by external factors. Theories of physiological polarity based on distribution or direction of flow of hypothetical "formative stuffs" or upon molecular polarity and orientation afford no satisfactory interpretation of the facts presented in this paper. On the basis of such theories we must assume that placing the animals in standing water or in the experimental solutions must alter fundamentally the distribution or direction of flow of the formative stuffs, or must alter the molecular orientation or polarity in many different ways. But there is not the slightest reason for believing that such changes in conditions could accomplish any of these results. In terms of physiological gradients, however, all the facts are readily and simply accounted for and brought into line with other facts, and the earlier observations concerning such transformations and changes in polarity are likewise easily interpreted. In this field, as in many others the gradient conception affords a basis for the interpretation and synthesis of data which has previously been lacking.

In view of apparently persistent misunderstanding of the gradient conception, it is perhaps necessary to emphasize once more the fact that it is concerned with the physiology of development, not with heredity. In other words, the specific protoplasm of *Bougainvillea*, of *Gonothyrœa*, or of *Obelia*, with all its hereditary potentialities, whatever these may be, is in each case the basis in which the gradient appears. This conception merely holds that, given this or any other specific protoplasm, the physiological gradient is an essential and fundamental factor in the realization of the hereditary potentialities in the form of an axiate individual of the species to which the protoplasm belongs.

The chief points are summarized as follows:

1. In various hydroid species the development of stolons can be induced by slightly inhibiting or depressing conditions, *e.g.*, low concentrations of ethyl urethane, $MgSO_4$, KNC, HCl, LiCl etc. and in most species even by change from natural conditions to standing water in the laboratory.

2. Stolon formation can be induced at all levels of the stock. The stolons may arise as adventitious outgrowths, by transformation of hydranth buds, or by transformation of terminal regions of stems after disintegration or resorption of hydranths.

3. These facts, together with data concerning the physiological gradients in hydroids indicate that the stolon axis is a somewhat inhibited gradient and less "steep" than the hydranth-stem gradient. The separation of stolons from the stock and the continued growth of stolon tips at the expense of lower levels in the absence of food are regarded as necessary consequences of the presence in the stolon of an axial gradient.

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THE DIGESTIVE SYSTEM OF THE PERIODICAL CICADA. II. PHYSIOLOGY OF THE ADULT INSECT.

CHARLES W. HARGITT.

The periodical cicada, now technically known as *Tibicen septendecim*, is well characterized by Marlatt (Bulletin No. 71, Bureau of Entomology, 1907, p. 11) as "undoubtedly the most anomalous and interesting of all the insects peculiar to the American continent. This cicada is especially remarkable in its adolescent period, the features of peculiar divergence from other insects being its long subterranean life of thirteen or seventeen years, during all of which time its existence is unsuspected and unindicated by any superficial sign, and the perfect regularity with which at the end of these periods every generation, though numbering millions of individuals, attains maturity at almost the same moment." Dealing with these several peculiarities and related problems of distribution, economic bearings, localized broods, etc., a large literature has accumulated since its first recorded advent at Plymouth, Mass., in 1623. But literature concerned with the more technical problems of its life history, for example, its embryology, morphology and physiology, is relatively small in volume and not of especially high value. Of some three hundred titles cited by Marlatt in the above-mentioned bulletin, by far the most of them relate to matters of habit, distribution, injuries caused, enemies, etc., and only an occasional reference to the anatomical or physiological problems concerned in its life. Much of this apparent indifference may be attributed to the highly obscure character of the life history of the insect, but not wholly so. For example, until the former paper by Hickernell appeared, there seems not to have been any critical account of the internal anatomy of any of the several organ systems of the insect. Nor has particular attention been directed to what must be rather unique physiological processes in such an organism. It is the purpose in what follows to submit an outline at least of the chief physiological observations which have been made, extending over a series of years, with an effort to correlate them with the clearly established anatomical and morphological features already described. In this connection will also be reviewed some physiological results of recent times which seem to have intimate bearings upon the immediate problems before us. While there has been intimate and continuous coöperation and mutual aid between the writers of this paper, in almost all phases of the research, it is frankly stated, however, that each is independently responsible for his own contribution.

The critical interest of the senior author in the morphology and physiology of this insect began over twenty-three years ago at which time its emergence in June, 1899, afforded a novelty in laboratory material which was presented before a large class in zoölogy. While in the external morphology of the insect there was ready recognition of the general equivalents, or homology, already familiar from laboratory studies of crickets and grasshoppers, it was quite otherwise when dissection was undertaken and a study of the internal anatomy was attempted. Here it was soon apparent that conditions were so different from anything before studied as to be puzzling in the extreme, and it was decided that this part of the subject was beyond profitable attempt at anything more than a general, and rather superficial survey, especially as it came in the hurried closing days of the college year. At a later time this problem was assigned to a graduate student, R. L. Henderson, reference to which was made by the junior author in his previous paper. The generally accepted view among entomologists was that this insect seldom or never feeds during adult life, and in part this led likewise to the view that the digestive organs were more or less degenerate or even atrophied. Such was my own conclusion from the preliminary study above referred to, and presented in a paper read before the County Academy of Science in whose proceedings the latter appeared in print. When Henderson undertook the work assigned to him just cited above, he was early forced to discredit my earlier conclusions on this point; and while unfortunately he did not live to complete his research, some of that which he left in manuscript shows that he had obtained clear evidence of the continuity and functional activity of the entire digestive system. The recent work by Hickernell has corrected and extended the investigation in a thorough and convincing way.

FEEDING HABITS.

A consideration of the feeding habits of cicadas is important in the present connection, as conditions in the digestive organs will be influenced by the reaction of the insect to food. As pointed out in the previous article, it has been maintained by various students of these insects that they seldom if ever take food during the adult life. Later studies by Quaintance, to which Marlatt has yielded a measure of assent, and the researches of the former paper already referred to, clearly establishing the functional efficiency of the digestive apparatus, renders the conclusion inevitable that there is no intrinsic difficulty in the views of these observers as to the feeding habits of the insect during the adult period of its life. The senior author has studied the food habits of this insect at several intervals for some twenty years and is not convinced that the accounts by Quaintance just mentioned are fully confirmed. Very special attention was given to this point during the appearance of the brood in this locality in the year 1916. In the main, it confirmed previous studies in 1899. In both cases attention was directed to two phases of the adult life, namely, that immediately following the emergence from the nymphal stage and during the early free-living condition, when it was believed the occasion to replace the wastes of this ordeal might express itself in free food taking. Furthermore, it was during this period of early adult life that accurate observations could be most easily made. But repeated observations at this time failed to show a single decisive case, though at times the attitude of the insect was such that it was necessary to disturb it in order to make certain that it was not actually feeding. Another period for observation was that about a week or ten days later when activity was very marked, especially among the males, in view of the growth of the reproductive organs and cells at this time. It was assumed that here was a special stage when need of food must be rather urgent. But here again, very few cases even under cage environment were distinguished. It must be admitted that during this period few specimens could be found in which these observations were easily studied and, as before stated, it was found very difficult to distinguish between a merely resting or quiescent state and that of the attitude of the insect in feeding without disturbing its pose. To make this a matter of precision, numbers of insects were placed in breeding cages in the laboratory. These cages were provided with fresh stems of shrubs, and twigs from trees, and thus kept renewed daily for some time. At the suggestion of the senior author, an out-door insectary was constructed upon the lawn by using mosquito netting which was spread over growing shrubs and carefully fastened to the ground. In this enclosure, scores of specimens were kept under close observation for many days. But while cases of apparent feeding were now and then observed among these specimens, upon closer examination it was found that actually a very few were feeding, comprising less than 1 per cent of those under these conditions of observation. In view of these results, it has seemed to us that one could hardly accept the contention of Quaintance already cited that there was "frequent and general" feeding during the insect's life. The senior author's field observations on this matter have been rather extended. He has never observed what has been described as the abundant "exudation of greater or less quantity of sap from the puncture" of such feeding specimens, or "the trunk and larger limbs quite wet with the sap which had escaped in this way," as described by Quaintance. These out-door studies were often made in the early hours of the morning when dew was dripping from every bush but it was not supposed that this was in any case an exuded sap of the plant tissues upon which the insects were found! But neither under these field observations nor in those carefully restricted to the inclosed insectaries already referred to, was the writer able, except in the rarest instances, to convince himself that feeding occurs at all; certainly it is not a *common and general feature of adult life*. Furthermore, numerous dissections made of the insects have failed to show the gorged conditions of stomach and rectum which is described by several as plethoric to the point of rupture on the slightest disturbance!

Feeding habits among other animals of similar life cycles confirms what has just been pointed out. It is well known that many other insects have similarly anomalous habits and life histories, and that among them are various species of ephemerids. From the early accounts of these insects so graphically described by Swammerdam ("Natural History of Insects," Eng. trans., 1758, pp. 103–27), on to the present time, it has been common knowledge that these and other species live as larvæ for many months or even two or three years an aquatic life. During this time, they are voracious feeders. Finally, at the time of metamorphosis, they emerge in enormous swarms during the summer, chiefly at evening, having relatively few hours of adult life, during which mating takes place and soon after the discharge of eggs, the early death of the adults. During this brief period of adult life, they take no food; the digestive system, and especially the mouth organs, being so imperfect as to render them incapable of active function. But like the cicada, these insects have the body tissue loaded abundantly with fat, which, in view of the extremely brief period of activity, can hardly be needed for nutritive purposes, but is doubtless utilized in the main for the rapid growth and perfecting of the generative organs and their products. I have verified these observations repeatedly and I am quite able to confirm what is more formally stated by Metchnikoff ("The Nature of Man," pp. 271–277). He shows that the rapid death following the act of mating and the discharge of eggs cannot be attributable to this act in itself since many males which have not undergone this action owing to the great excess of male insects yet die as promptly as do others which have participated in the process. He also shows that death cannot be due to the presence of pathogenic organisms since diligent search has failed to reveal their presence; and further that it is not due to phagocytic action, since the organs show no indication whatever of such invasion. He suggests the probability that such rapid death may be the effect of the early death of the cells of the nervous system, yet gives no evidence in support of the suggestion. In a later work, "The Prolongation of Life," this author emphasizes the significance of natural death in many groups of lower animals and the unique modes of providing against its hazards to the continuity of the species. Among these, he cites observations and experiments upon Rotifera (pp. 113, 118). "The whole course of life from the laying of the eggs until death lasts only about three days and is probably the shortest duration of life in the animal kingdom. . . . The little males begin to swim soon after hatching, the wheel apparatus and the musculature being vigorous. They seek out the females, as their reproductive organs are mature at the moment of hatching. The transparent body, which is devoid of digestive apparatus, swarms with mobile spermatozoa. As soon as the male has seized the female, he discharges the contents of his body. It might be supposed that such an evacuation would cause violent perturbation of the system leading to the death of the organism. But the males are able to live for many hours after having accomplished their function, and the period represents a third of their natural duration of life. Moreover, I have isolated males from the females without any prolongation of their life. In one experiment, I isolated two males and placed a third in company with two females. It was the third specimen that lived longest. There can be no doubt but that the death of these male rotifers is natural in the fullest sense. The females, although they are provided with complete digestive organs, do not escape a similar fate. In some Ephemeridæ, which supply good cases of natural death, the end comes after a few hours of adult life without any sign of degeneration of the organs. As in others (Chlöe) life lasts several days without food ever being taken, it is clear that inanition is not the cause of the swift arrival of death in the first set."

As will be perceived, these citations from Metchnikoff relate primarily to distinctly different problems. But they are not without a measure of significance in connection with those under review. One point of importance is the fact that in these widely differing groups of organisms certain very fundamental functions, especially that of reproduction, take place normally during a period of inanition. Granting that the phenomena related of the Rotifers may be somewhat exceptional, and of only incidental significance, certainly those exhibited by the Ephemerids are clearly significant and pertinent, and have much in common with those so conspicuous in the life history of *Cicada;* thus accentuating the occurrence of kindred phenomena in widely differing organisms.

Fat Storage in Animal Economy.

The phenomenon of storage of fat among animals is a fact very well, and long known, and its physiological significance has been also generally recognized. Its occurrence in animals which pass long periods of hibernation, during which no food is taken, hence are dependent upon those reserve sources for sustenance, is a matter equally well known and common in many groups of animals. Among these are mammals, reptiles, amphibia and fishes. In the last group are cases in which such reserves are accumulated to meet extra and unusual demands which are involved in extended migrations to distant spawning grounds. And further dependence upon this store of energy is required for the maturation and fertilization processes involved in the reproductive crises common to many of this class of animals. This phenomenon is known in numerous species among which the salmon is a familiar example, with the rather tragic consequence that this climactic performance is usually followed by immediate or early death of the organisms.

Some recent investigations and experiments of rather striking importance have been made by Prof. C. W. Greene concerning the physiological processes, both of the storage of this reserve material and its later resorption by the tissues. (Bull. U. S. Bureau of Fisheries, 1914, pp. 73–138.) Professor Greene has studied this especially in the King salmon during the long fast of the spawning migration. He shows how the storage takes place in the musculature and connective tissues during the late growth, and especially the voracious feeding just prior to the migratory ordeal which involves hundreds of miles up great rivers and against many and serious obstructions. The energy consumed during this ordeal must be supplied by these reserve sources of nutrition. And as just pointed out, the additional demand involved the growth and ripening of the sexual products and actual spawning of these at the end. It will be at once perceived that for such an ordeal very large resources of energy must be available and these are to be found almost wholly in these reserve fat materials. Many other such experiments have been made upon various species of animals such as amphibia, reptiles, etc., all going to sustain the above cited findings;

namely, that the storage of potential energy in the form of fats or surplus proteids is an obvious provision for maintenance of vital functions during periods of reduced or suspended activity, but which is made available by a reversed metabolism, brought about by the operation of identical or similar enzymes, as shown below. Greene has, by actual experiments and extended observations during these migrations of the salmon, shown with clearness and convincing results the entire physiological history of the absorption and storage of fats, and its later transportation to the various tissues and organs concerned. He also critically reviews the earlier work of Miescher along these lines and emphasizes its values, at the same time showing certain of its defects, especially its erroneous contention that the fats found in muscular tissues were degeneration products; and shows convincingly that the presence of fat in such tissues is a result of infiltration and "that intracellular fat of the King salmon is an expression of the nutritive state of the muscle. It is a loading of fat by a process of infiltration and is not a degeneration of the muscle substance." He next points out the applicability of the same discovery by Kastle and Lovenhart of the reversible action of lipase and, as a consequence, gets an insight into the mode of transportation of fats from tissues to tissues in the animal body. (*Ibid.*, pp. 123–125.) These researches of Greene throw fresh light upon very similar problems of reserve energy of storage fats well known in invertebrates. For example, the cases of the Ephemerids, Lepidoptera, and the periodical cicada, all of which show points of close similarity to the foregoing. Among insects, this reserve material is accumulated chiefly in a peculiar organ known as the "fat body" which is "of various shapes," according to Packard, "more or less lobulated and net-like and covers parts of the viscera, also forming a layer under the integumen. The tracheal endings are usually enveloped by the fat body. It is larger in the larvae than in the adults, especially in Lepidoptera, in them forming a reserve nutrition used during the metamorphosis and during the formation and ripening of the eggs and male cells." (p. 419.) According to Wheeler whom Packard quotes, this fat body is of mesodermal origin, and differentiated from portions of the cœlomic walls, hence of metameric origin. Numerous more or less conflicting accounts have been given as to the particular function of this body. For example, Marshal regarded it as a urinary organ; Graber regarded the entire system of the fat bodies as a simple many-lobed lung; a view likewise taken by Landois; but Schäffer took the view, now generally held, that it is a reservoir of nutrition from which the organism may draw during times of special stress or emergency. The case of the cicada is peculiar; for its whole larval and pupal existence comprises from thirteen to seventeen years of underground life devoted especially to feeding and growth. These finally culminate in the crisis of reproduction which lasts only two or three brief weeks. But during these weeks, feeding is almost wholly lacking as has already been previously shown. When first emerging into adulthood the body of this insect is literally gorged with storage fat and related reserves. But these rapidly decrease with growth and development of the reproductive cells, and with the maturation and discharge of these cells, this reserve supply becomes rapidly exhausted, especially in the male, and the female completes its exhaustion in the arduous task involved in puncturing branches and twigs for receptacles in which the eggs are laboriously deposited. These functions completed, the vigor of the insects rapidly declines, since the storage being exhausted and taking no new supplies, they rapidly decline and die. As an interesting incident bearing upon the matter, may be stated the fact that in the use of these insects as an article of food, which is common among American Indians, they are taken exclusively at the time the insect emerges in its mature form, or at least, very soon after, for at this time this storage matter is at its best and later, of course, rapidly deteriorates. At this period, also, the insects are preyed upon by hogs, fowls, and such birds as feed upon them, since only at this time are the insects easily available. For birds, they can be taken during the entire life period of a few weeks, but naturally, are most sought in early life when they are more easily captured.

There are many analogous features between the physiology of fat storage as shown in the foregoing citations from Greene's experiments and what probably takes place during the life cycle of these insects, some before mentioned. Among others the following are of interest.

1. The relatively long and probably more or less continuous

periods of feeding and growth. Experiments show that for the salmon it may be five to eight years; for the ephemerids two or three years; while for the cicada thirteen and seventeen years. In these groups this long period is now believed to be generally concerned in accumulating reserve potential energy, most of which will sustain an important relation to the brief, but crucial period of activity and perfecting the reproductive elements, and their union for the preservation of the species.

2. In each of these groups this actively cumulative growth and storage of energy, followed by a relatively brief period of reproductivity, gives that anomalous reaction of decline and death a unique significance.

3. Corresponding to these extended and painstaking researches of Greene I know of nothing among insects or other invertebrates; but the remarkably analogous aspects of the cases lead me to conclude that the physiologic activities involved in the latter are more or less similar; in some respects identical, with the former.

The Posterior Crop.

Under this caption, the junior author in his earlier paper has described a most unique and anomalous organ, clearly, as I believe, a part of the digestive system. For a full account of its anatomy reference may be made to his description in the paper just cited. It must suffice here to briefly summarize its main features. During nymphal life, as is well described in the second section of this contribution, it is rather small, "with walls of uniform texture and much folded. But in the adult, the walls of the organ are distinctly variable in thickness. The outer surface of the organ is closely apposed on all sides by fat. This probably has something to do with the collapsed condition of the tube in this region." A recent popular paper by Snodgrass descriptive of this insect designates this organ on the contrary as a part of the respiratory system. But, as will be shown later, this seems decidedly erroneous. In my earlier account (Proc. Onon. Acad. Sci., 1903, p. 51) I conceived it in adult life to act in some way as an organ for aiding in the absorption of fat, its epithelium in many cases being more or less charged with globules of fat. This was confirmed by the work of Henderson who also showed that in no case of his numerous dissections, more than two hundred in all, of either nymph or adults, did he find traces in this organ of undigested food.

All this is abundantly confirmed by my own later work as it in turn also confirms earlier observations as to the feeding habits of these insects. Likewise, this is found borne out by the microscopic sections of the canal through every region of the mature insect by the junior author as shown in his previous paper. But there are certain rather puzzling features in this particular organ in adult life. It does not appear clear that its increase in size at this time can be due to a reservoir function, unless feeding be increasingly active during later life, which certainly does not seem to be the case. Again such a view seems to be in direct conflict with the histological character of the organ which shows clear evidence of degenerative changes in its lining epithelium. On the other hand it seems to conform with the view just previously expressed that it is during this stage apparently functionless in any active way, and that as the storage elements are resorbed the organ reacts in consequence, its walls expand to occupy the visceral spaces which earlier were filled by storage matter and reproductive organs, which in turn accounts for the attenuated condition of the epithelium described by Hickernell as above stated.

A further fact in this connection remains to be noted, namely, that these adult insects leave no signs of excretory wastes, such as defecative products. The writer has handled living specimens by hundreds, taken at various times, some kept under bell jars, others in clean breeding cages, as well as others still which were freely handled, but has not seen at any time evidence of defecation. Of course, the liquid sap upon which these insects feed might, and doubtless does show less of solid waste to be discharged, but certainly there are unused elements even in such foods as sap which doubtless are extruded; as are those heavier products whose wastes are so conspicuous in the life surroundings of most feeding insects. The well-defined and thick-walled rectum of the cicada goes to support this view. This point is made as a matter of fact which should not be overlooked; but so far as I am aware it has not been given attention heretofore. Were feeding at all frequent or general in the adults such solid excreta could hardly fail of notice. Their absence, therefore, can hardly be other than highly significant of the lack of active digestive operations during adult life, and is in entire accord with the fact of the entire absence of alimentary elements in the tract as above cited.

During the progress of this work my attention was called to a popular paper by R. E. Snodgrass on the Seventeen Year Locust (Smiths. Rept. for 1919, Washington, 1921, pp. 381–409), in which there appear certain views rather sharply in conflict with those herein maintained, and which call for some brief attention. Its anatomical points are reviewed by the junior author in the section which follows. But it falls to me to notice phases of feeding habits and others of a physiologic nature. In reply to certain inquiries submitted to Snodgrass he was kind enough to write me quite freely as to the questions, and also sent a specimen of transected insect to show the highly cavernous aspects of late life, and to afford what was suggested as a demonstration of the tracheal nature of the so-called "air-chamber." This, I examined with care, but cannot accept as demonstrative, since there were no distinct evidences of its tracheal structure, as the junior author conclusively shows in the histologic demonstrations of all phases of the typical and deteriorative epithelial cells occurring, and an entire absence of chitinous elements or tænidia in the organ.

Of this "airchamber" Snodgrass states that it "receives its supply of air directly through the spiracles of the first abdominal segment." If this were so there should be unmistakable evidence of its being a paired organ, as in the bee; but of this there is no evidence whatsoever, a fact which he admits but claims such to be the case in the dog-day cicada. This I have not been able to discover from actual dissections, or from serial sections of the regions, as shown by Hickernell in the earlier paper.

Referring to its function as related to the respiratory system Snodgrass without hesitation discredits the view of Graber, that it may have some relation to the tympanal organs, but says, "We shall probably have to fall back on the old prosaic explanation that bulk of body is maintained with corresponding weight eliminated—a combination specially favorable to aërial life." But as Packard long ago pointed out (cf. p. 457), this assumption is erroneous, "The body of the insect during flight not being lightened by the air in the sacs." Submitting this point to two of my colleagues of the department of physics, Doctors Porter and Packard, I am assured that it is entirely correct.

It seems rather certain, therefore, that neither from its structure, nor yet from the "old prosaic explanation" of an adaptation to aërial life, does he sustain his conclusions. Granting the ramifications of tracheæ over the organ no more makes it respiratory than does a similar disposition of tracheæ over the viscera or the musculature constitute them such. Apart from the names by which Graber designates the organ, namely, "Tracheenbläse" and "Luftsäcke," there is nothing in his account which supports the views of Snodgrass. Graber's problem was the "Tympanalorgane," not respiration. I believe therefore that Snodgrass has misconstrued Graber's work so far as it is applicable to the matter at issue, and that his view may be dismissed as devoid of structural or functional evidence as a respiratory organ, or as an accessory "Tympanal organ," as claimed by Graber (p. 282–3).

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THE DIGESTIVE SYSTEM OF THE PERIODICAL CICADA, TIBICEN SEPTENDECIM LINN.

III. MORPHOLOGY OF THE SYSTEM IN THE NYMPH.

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In an earlier paper, the form and relationships of the various parts of the digestive system of the adult cicada were considered. It was found that in both sexes the system is complete and well organized but much complicated by a winding and twisting together of its parts, so that the continuity of the system could be established only with some difficulty. The intertwining intestine, esophagus, and malphigian tubules in the anterior part of the body cavity makes the "internal gland" or filter which seems to be characteristic of so many of the Homoptera. This structure, while different in some respects in the cicada from that described in other homopterous forms, has a general arrangement which is similar.

It was found further that certain organs of the digestive system change shape markedly, as the insect increases in age, but that the ground plan of the alimentary tract remains the same throughout the life of the insect and that the system does not degenerate or become broken as has been believed by certain workers.

Because of the numerous peculiarities which were found in the adult digestive organs, it seemed worth while to examine the nymphal stages with the object of determining what conditions exist there and also to determine, if possible, the origin of some of the peculiar structural relationships. For, although the peculiarities of the homopteran digestive tube have been recognized for some time, the beginnings of these peculiarities in embryological history have not been figured or explained in many cases. It can at least be established whether the peculiar windings of the digestive organs arise at the time of one of the numerous moults or are to be traced back to the egg itself.

The consideration of the morphology of the digestive organs of the Homoptera dates back to the work of Lubbock, Leydig, and Ramdohr about the middle of the last century. Lubbock described the digestive organs in *Coccus hesperidum.* He observed the complicated windings in the anterior region and called this complex the internal gland.

Dufour (1833) described the digestive apparatus of certain Hemiptera but the significance of some of the structures which he described apparently escaped him. In fact, it has been only recently that the digestive systems of any great number of Hemiptera have been worked upon and the true nature of the structural peculiarities of the organs determined.

Witlaczil ('85) studied the anatomy of the Psyllidæ. In *Psyllopsos fraxinicola* the digestive canal has many of the peculiarities which have been noted in the cicada.

Berlese (1909) describes the digestive organs of certain scale insects. In these insects the rectum is large and extends anteriorly as far as the esophagus. This results in a knitting together of rectum and esophagus and also causes the intestine to describe a complete circuit of the abdominal cavity before it finally joins the rectum. This condition, while resembling it superficially, is entirely different from the arrangement found in the adult cicada since in the latter the enormous enlargement affects the mid-gut while the rectum is relatively small.

Licent (1911) gives an account of the digestive organs in the Cercopidæ. In this family there is also a loop made by the mid-gut which curves backward so as to become intimately associated with the anterior part of the canal. Licent believes with Berlese that this complication acts as a filter, allowing the watery part of the food to diffuse directly through the walls of the fore-gut into the cavity of the hind-gut and in this way saves the mid-gut for digestive activity upon the more concentrated food mass. The nutritive substances, greatly diluted with sap, are thus concentrated and, for the most part, digested in the mid-gut.

Kershaw (1913) recorded his observations on the digestive organs in *Siphanta acuta*, a flatid. This form has a large reservoir or crop, which from its junction with the esophagus just within the abdomen, extends anteriorly above the esophagus through the thorax and practically fills the epicranium above the brain. It also extends posteriorly beneath the heart and above the rest of the internal organs, almost to the tip of the abdomen. It is interesting to note that this food reservoir is similar in most respects to the enlargement in the digestive tube of the cicada, and furthermore that it attains its enormous dimensions only in the later adult life of the insect. In this last respect it further resembles the condition in the cicada.

From the above accounts taken with other works not quoted here, it seems evident that the digestive systems of the related forms of the group under consideration studied to date have, in general, the same ground plan and that their specializations although varying in degree, are mostly of the same kind. These structural complications have led to different interpretations of the relationships and functions of the various organs, but it seems likely that there is a much closer similarity in these respects than at first appeared to be the case.

The conclusions here presented are based upon a study of nymphs of *Tibicen septendecim* Linn. of four different sizes, viz. 2½ mm.; 7 mm.; 14 mm.; and 21–24 mm. This series covers practically the entire period during which the insect lives under ground. The 2½ mm. nymphs are only recently hatched from the egg, while the 21–24 mm. individuals were full grown nymphs dug from the ground about one month previous to the general emergence and transformation of the brood. According to Marlatt, the nymphs of 7 mm. body length are about four years old, while the 14 mm. ones are about ten years of age. These relationships between size and age were established by workers in the Bureau of Entomology, who followed a complete life-cycle of one of the cicada broods.

The method of serial sectioning and reconstruction was followed throughout this study. The impossibility of tracing the alimentary canal by means of gross dissections is even more apparent in the immature forms than it is the adult.

In describing the digestive organs as found in cicada nymphs of different ages, it would be more logical to begin with the youngest and follow the developemental series until the adult condition is reached. However, in an earlier paper the adult digestive organs were described and the descriptions and comparisons here given have been written keeping the adult structure in mind. Obviously, in such a situation it will be easier for the writer and more understandable for the reader to work backwards through the series of nymphal stages where structural variations are relatively slight, as between two successive stages, than to jump from the adult condition back to the earliest nymph and then work up to the adult condition once more. It is believed that a description of these different stages in reverse order is warranted in the present instance in view of the circumstances outlined above.

The alimentary tract in nymphs of 21–24 mm. length has the same general arrangement of parts as is found in the adult. The form and structure of the individual organs varies, however, in some respects. The posterior crop (Fig. 1, *pc*) always has its walls wrinkled and contains a tortuous lumen, as if the structure had collapsed completely, while in the adult this division possesses smooth, thin walls surrounding an enormous lumen. The size relations of some of the other parts also vary as is shown hereafter. A general view of the entire system as seen from the left side is represented in Fig. 1. The esophagus is a simple tube, uniform in diameter, and leads into the anterior crop (*ac*). The latter with its dorsal adherent "internal gland," is of about the same relative size as it is in the adult. The anterior crop empties through a narrow opening into the posterior crop (*pc*). The latter is greatly unlike the corresponding division in the adult. Its relative length is much the same but its walls are uniform in texture and much folded throughout. In the adult the walls of the organ are distinctly variable in thickness and are not folded to any extent except at the extreme anterior end where the cæcal projection runs ventral to the anterior crop. The outer surface of the posterior crop is closely apposed on all sides by fat. This probably has something to do with the collapsed condition of the tube in this region. At any rate, its opposing walls almost touch each other throughout its whole extent, thereby making the lumen narrow and irregular (Fig. 4, *pc*).

The ascending intestine is relatively larger in diameter in the 21–24 mm. nymph than it is in the adult. Its general course and connections are the same as in the adult but its size, both externally and with respect to its lumen is noticeably greater (Figs. 1 and 4, *at*). The ascending intestine enters the internal gland in the same fashion as it does in the adult. The descending intestine also emerges from the complex of tubes as it does in the fully developed insect.

The descending intestine is relatively smaller in this stage than it is in the adult condition. Emerging from the internal gland it runs in a general posterior direction as in the adult, finally making a knot or coil (Fig. 1, *kk*) just before emptying into the rectum. There are some variations in the histological structure of the epithelium in its walls but the tube is easily recognized in section when one is familiar with its microscopic structure in the adult.

The rectum does not differ greatly from that in the transformed insect. It receives the descending intestine and then gradually narrows until the anal opening is reached.

Among the younger nymphs there are variations in size and arrangement of the digestive organs but these are slight as compared with the structural differences shown between the organs of the early nymphs and those about to transform to the adult condition. In nymphs of 14 mm. and 7 mm. body-length there is not enough variation in the arrangement of the organs in the two stages to warrant separate description. Figure 2 is based upon specimens of 14 mm. length but, except for size, the same figure applies to the shorter and younger stage. Esophagus, anterior crop, and internal gland are practically the same as in the stage previously described. The remaining portions of the system, however, differ greatly in many respects.

The posterior crop does not have any suggestion of saccular structure in these early stages. Its walls are folded as in the other previously described specimens but it does not assume the enormous diameter common to that division in the older nymph and adult. In fact, the ascending intestine has a larger diameter in this stage than does the posterior crop. Its course is almost straight through the center of the body until it reaches a point just anterior to the rectum. Here it joins the ascending intestine.

The ascending intestine (Fig. 2, *at*) is exceedingly prominent at this time. It is much convoluted and extends posteriorly in the region of the rectum, from which place it runs anteriorly. For the most part it runs along the ventral surface of the body and when it reaches the internal gland it disappears as in the adult. The interweaving of the posterior crop and the two intestinal divisions seems difficult of interpretation when first studied in sections. However, the general ground plan is soon seen to be in no way different from that in the stages previously described.

The internal gland has the same structure here as it does in the older nymph. In size, it is of course, smaller but sections show identical parts in the two stages.

The descending intestine and rectum have the same form and arrangement in the 7 mm. and 14 mm. nymphs as they do in the later stages. The diameter of the descending intestine is very small. It runs close to the dorsal integument in some places and might easily be missed in studying sections. The characteristic coil (Fig. 2, *kk*) exhibits a convolution which is practically the same as that described for the later stage.

Examination of Figs. 1 and 2 makes it clear that there is no great difference in the arrangement of the digestive organs of the nymphal stages considered. It is also true that in the $2\frac{1}{2}$ mm. nymph there is not enough difference in structure or arrangement to warrant making a separate figure to represent conditions there. This means that the plan of the digestive apparatus is not altered practically throughout the entire underground existence of the insect. The variation in the size of certain organs at different periods in the life-history suggests either that the nymph does not feed continuously or that there is a change in the function of some of the organs as time goes on. The former supposition is probably correct for it is known that these immature forms have alternate periods of feeding and resting.

The ascending intestine is found in some sections to have its epithelial lining made up of enormous cells filled with granules. In other cases we have the condition represented in Fig. 4, *at*, where the walls of this organ are thin and attenuated. These variations probably represent different phases of functional activity and are not to be interpreted, therefore, as indicating any change in the plan of digestive activity.

The complication of organs in the anterior region which has been called the internal gland, arises at a time earlier than that represented in the stages here described. Fig. 3, which is a transverse section through the internal gland region of a 24 mm. nymph, shows all the parts arranged in a manner similar to that in the adult. In Fig. 5, which is a like section through the same region in the 14 mm. nymph, the same organs are found as before, but there is slightly less complication in the way of folding and intertwining than is found in the later stages. Figures 7 and 8 represent transverse sections through the anterior region of the digestive organs of a 2½ mm. nymph. Here again the various organs are seen to have assumed a position similar to that in which they are found in the later nymphs and adult. The peculiar relation of anterior crop, intestine, and malphigian tubules is, therefore, established in all these nymph stages the same as in the adult. In seeking the origin and significance of this arrangement, it is necessary, then, to go back to the development of the embryo within the egg.

In Figs. 1 and 2 the malphigian tubules have not been represented. They are present in the same number and arrangement as in the adult. They have been left out of the above figures since they only tend to obscure the clear representation of the digestive organs.

The function of the posterior crop as an accessory storage organ seems, at first, to be indicated by a comparison of the different stages here considered. Originally a tube of small diameter, it increases in size until it exceeds any of the other organs in capacity. I have never found any precipitate or coagulum in the cavity of this organ, however, so that the mere size of its lumen may not justify one in attributing a storage function to it.

In summarizing it may be said that from the observations upon the four nymphal instars of the cicada it is evident that the digestive organs show an arrangement which is similar in ground plan with that of the adult and also it is similar in many respects with that of other Homoptera which have been described. The complication of digestive organs in the anterior region of the insect is fully established in the earliest nymph and hence is developed at a time previous to that represented in the material here considered. The posterior crop loses its simple tubular character and becomes saccular at some time after the 14 mm. nymph stage.

Since the foregoing part of this paper was written, the paper by Snodgrass dealing with the anatomy of the cicada has come to hand. In this publication the large vesicular organ which occupies the greater part of the abdominal cavity in the adult insect and which I have called the posterior crop, is considered as a part of the respiratory system. The chief reason for this is the apparent continuity existing between this sac and the first pair of abdominal spiracles. Mr. Snodgrass has been kind enough to demonstrate dissections to me which seem to bear out his contentions. There are, however, some fundamental objections to his position.

In the first place, a "tracheal bladder" or respiratory duct of any kind in an insect should show a lining layer of chitin since the tracheal system of insects arises as an invagination of the primitive ectoderm. In a former paper sections of the "tracheal bladder" of Snodgrass through three different regions were shown and none of these showed any evidence of a chitinous layer. It has been suggested that perhaps this structure attained its respiratory function secondarily and hence might not conform in all structural details to expectations. It is hard to imagine how an entire segment or organ of the digestive tube could undergo such a transformation of function.

In my sections also, I have shown that there are distinct openings at the anterior and posterior ends of this organ, the one at the posterior end leading into a continuation of the digestive tube and that a muscular valve intervened between these two divisions. In view of this evidence it is difficult to conceive of this part of the abdominal contents as having a respiratory function.

It is easy to be deceived as to the continuity of the lumen of the posterior crop with the exterior through these first, abdominal spiracles. In gross dissections there is only the most delicate epithelial membrane limiting this abdominal sac in the region of these spiracles. In my earlier paper a figure was shown covering this point. If a specimen is allowed to become dry, the portion of the wall which is in front of the spiracular opening may easily rupture and then there is an external opening in fact.

Sections show that the spiracle opens into a very small chamber the walls of which break up almost immediately into a number of tracheal tubes which distribute themselves *over the external surface* of the posterior crop. I am therefore, still inclined to question any interpretation which gives this organ a respiratory function. It certainly becomes modified in later life but it is at all times a part of the digestive system. This condition which is easily observed in sections makes unnecessary the postulation of any secondarily derived function on the part of this organ. The method of gross dissection, then, is inadequate to explain the conditions found.

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EXPLANATION OF PLATES.

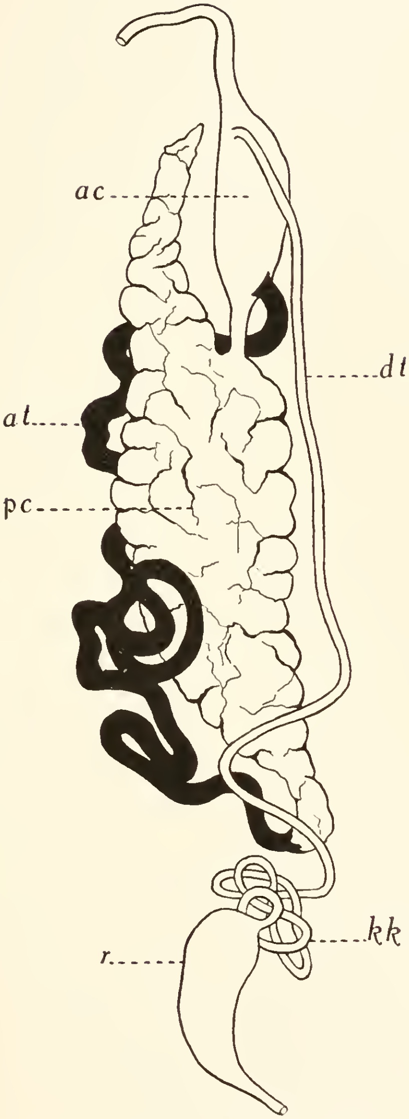
INDEX TO FIGURES.

ac—anterior crop.
at—ascending intestine.
dt—descending intestine.
int—internal gland.
kk—coil in descending intestine.
mt—malpighian vessels.
pc—posterior crop.
r—rectum.

PLATE I.

FIG. 1. Digestive system of cicada nymph of 2 mm. body length, seen from the left side.

FIG. 2. Digestive system of cicada nymph of 14 mm. body length, seen from the left side.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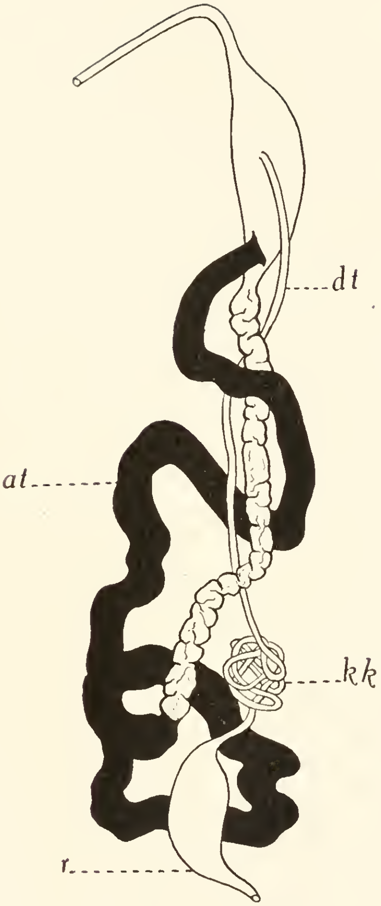

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Plate II.

Fig. 3. Transverse section through the internal gland region of a 2 mm. nymph.

Fig. 4. Transverse section through digestive organs in anterior region of a 7 mm. nymph.

Fig. 5. Transverse section through internal gland region of a 14 mm. nymph

Fig. 6. Transverse section through digestive organs in posterior part of a 7 mm. nymph.

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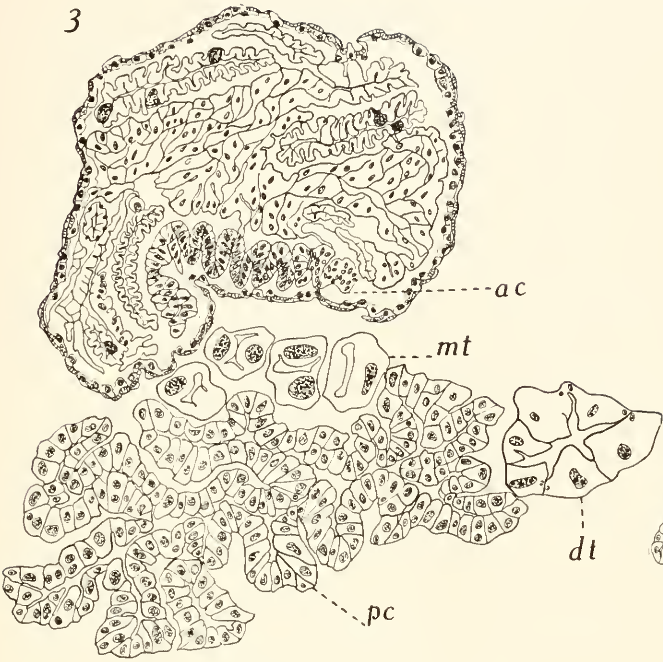

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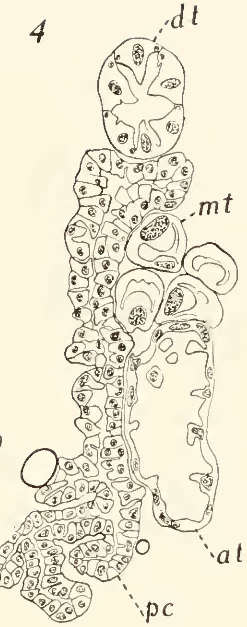

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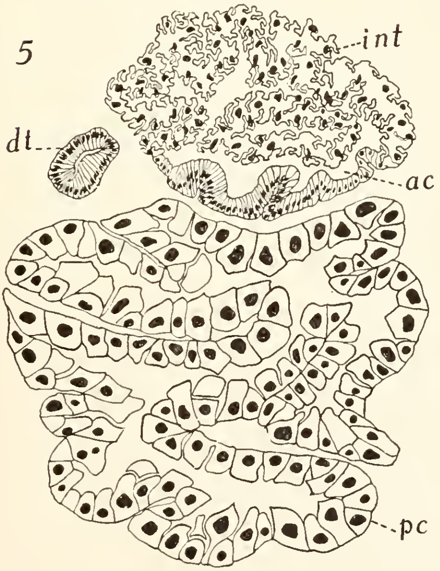

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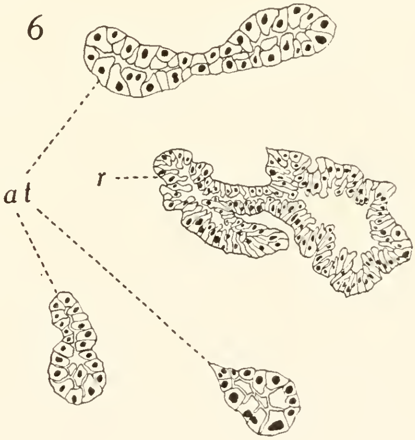

PLATE III.

FIG. 7. Transverse section through anterior part of digestive organs of a nymph recently hatched.

FIG. 8. Transverse section through same region but slightly anterior to that represented in Fig. 7.

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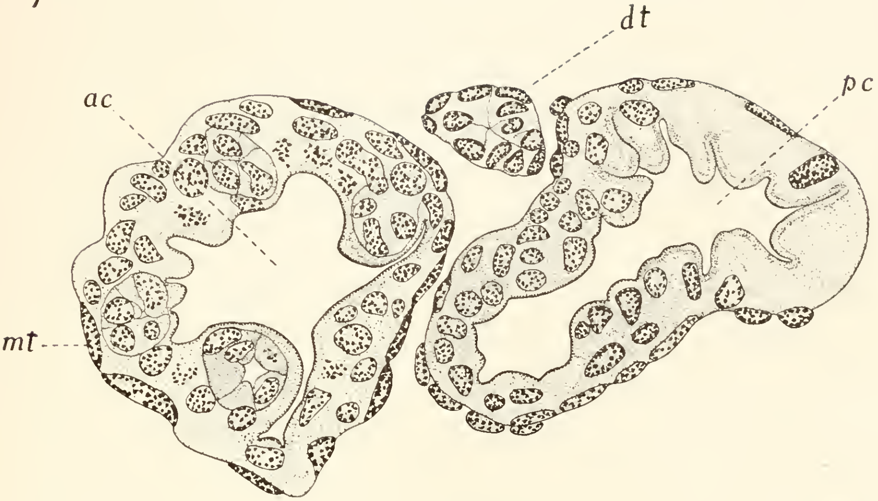

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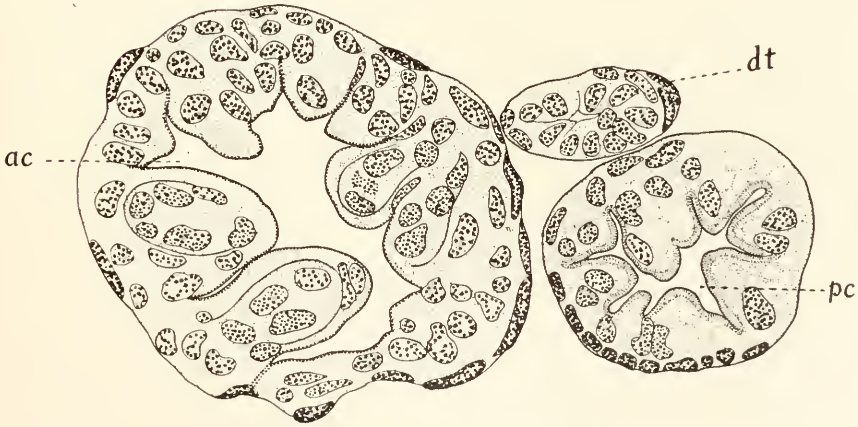

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PLATE IV.

FIG. 9. Transverse section through digestive organs in mid-body region of a nymph just hatched.

FIG. 10. Transverse section through posterior part of digestive organs from the same series as Fig. 9.

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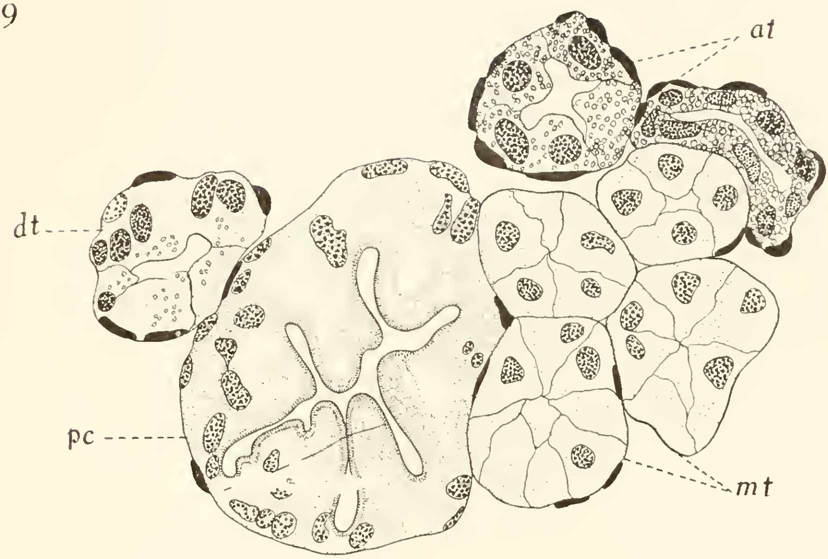

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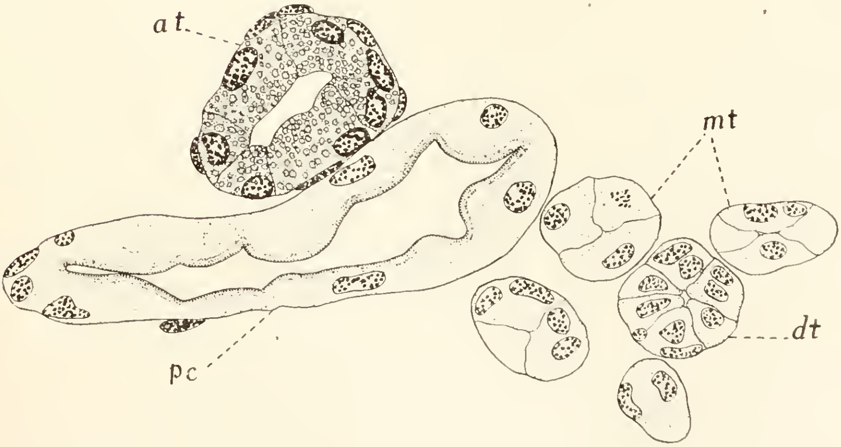

L. M. HICKERNELL.

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BIOLOGICAL BULLETIN

IODINE AND AMPHIBIAN METAMORPHOSIS.[1]

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Recently the claim has been made that the metamorphosis of urodele larvæ such as axolotl and amblystoma differs from that of anurans in that transformation in the caudate amphibia is independent of iodine and subject only to the influence of the thyroid gland. Experiments are described in this paper which invalidate this conclusion and indeed, render it impossible to understand such a view. The rôle of iodine in metamorphosis is also discussed, and evidence presented showing that the physiologic response (metabolic changes) of mammals to thyroid administration and the metamorphic changes of amphibian larvæ following iodine treatment are probably due to quite different causes and hence not to be compared.

Acknowledgement is due Mr. William Anderson for his labor in iodizing the various compounds employed.

The axolotls came from New Mexico, and were obtained through the courtesy of Mr. J. N. Gladding of Albuquerque.

I. Experiments on Thyroidectomized Axolotls.

The thyroid glands of eight animals were extirpated. One larva was extremely large, measuring over a foot in length. The remaining larvæ averaged seven inches total length. Few technical difficulties are involved in removing the thyroid glands from animals as large as the axolotl. The larvæ were anesthetized in chloretone solutions, placed upon their back under a low-power binocular microscope, and the hyobranchial region strongly illuminated. A median incision was made through the skin and superficial muscles, extending from the posterior edge of the hyobranchial region to the symphysis of the mandible. The skin and superficial muscles were pulled apart on either side and pinned down, exposing the geniohyoid and ceratohyoid muscles. The thyroid glands are located in the triangle on each side of the median line, formed by the geniohyoid and ceratohyoid muscles. The large size of the glands and their intimate relation to the blood vessels which traverse the triangle render their location easy. They can be removed from both sides of the animal with but little injury to the blood vessels if first dissected free with a razor-edge needle and gently pulled out with a fine-pointed pair of forceps. Even if the blood-vessels are injured and considerable bleeding occurs, the post-operative effects upon the animal are negligible. It is surprising how much surgical manipulation an axolotl can withstand without showing any post-operative symptoms. Following gland removal the skin and superficial muscles are sutured along the median line. The animals quickly recover from the anesthetic and appear to suffer no ill effects from the operation. Food was witheld for four or five days after thyroidectomy in order to prevent any possibility of tearing the sutures while swallowing.

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[1] Part of the expense of this investigation was defrayed by a grant from the Elizabeth Thompson Science Fund.

The thyroidless animals were kept for five months in large concrete tanks through which fresh water ran constantly. The food consisted of worms, insect larvæ, and occasionally, pieces of fresh liver.

Five months following thyroid removal, three animals were injected twice, at five day intervals with eighty milligrams of tyrosine in which two atoms of iodine had been substituted for two hydrogen atoms of the molecule, forming the well-known compound 3-5-diiodotyrosine. The animals metamorphosed within seventeen days following the first injection.

Three control thyroidless axolotls injected with equal quantities of pure tyrosine and 3–5 dibromtyrosine, *i.e.*, tyrosine in which two bromine atoms had been substituted for two hydrogens, failed to transform. Later, one of the controls was injected with a third dose of eighty milligrams of dibromtyrosine but with negative results. Dissection of the metamorphosed iodotyrosine-injected animals showed no trace of thyroid tissue present.

Rogoff and Marine ('17) showed that iodized blood serum accelerates metamorphosis of normal tadpoles, and that the globulin fraction of the serum contained most of the iodine. It was decided to try injecting iodoserumglobulin into thyroidless axolotls, controlling the experiment by injections of equal amounts of non-iodized globulin. The blood of beef was used.

Three axolotls were each injected twice, at eight-day intervals with 110 milligrams of iodoserumglobulin. Metamorphosis resulted within twenty days following the first injection. One of the axolotls had previously served as a control in the iodotyrosine experiment and had been injected with pure tyrosine. The results were negative and as the larva showed no indications of metamorphosis several weeks later it was utilized in the iodoserumglobulin experiment. Two control thyroidless animals (previously used as controls in the iodotyrosine work) injected with large amounts of non-iodized serumglobulin failed to transform.

Dissection of the metamorphosed iodoserumglobulin-injected animals showed two of them to have no vestige of thyroid tissue, but the remaining one had a portion of the gland present on the left side, an amount about equal to a third of the entire thyroid. One of the controls was then injected with iodoserumglobulin and metamorphosed twenty-one days after the first injection; dissection of this animal also showed a small nodule of thyroid tissue present. Thus four axolotls were metamorphosed by injections of iodized serumglobulin; two of the animals were completely thyroidectomized, and two only partially. No very marked difference in the time or rate of metamorphosis was noted between the partially and completely thyroidectomized animals. The axolotls possessing remnants of glands were the first to show signs of transformation following injection, but the time difference was not over three days in any case. The fact that both types of animals metamorphosed within a few days of each other may have been due to the large amount of iodoserumglobulin injected. It is probable that partially thyroidectomized animals will be found to respond by metamorphosis to considerably smaller doses than thyroidless animals. Axolotls were not available to test this point.

Considerable difficulty was experienced injecting the globulin intraperitoneally. The solution of the problem while crude was effective. A small incision was made through the ventral body wall sufficiently large to admit the end of a graduated pipette and the globulin was injected in powdered form. This method because of the ease with which it could be performed was also utilized in the tyrosine experiments. Forced feeding of the animals with the various substances by means of a pipette thrust down the œsophagus into the stomach, might have been as effective as injections, but the animals usually regurgitate some of the material, at any rate this was found to be the case in an earlier experiment where desiccated thyroid tissue was administered by forced feeding.

The pigment pattern characteristic of adult Amblystoma tigrinum is not completely developed in axolotls until several weeks following metamorphosis.

Jensen ('21) metamorphosed axolotls (with intact thyroid apparatus) by injections of iodized casein, iodoserumglobulin, and iodoserumalbumin. He also removed the thyroid glands of axolotls and attempted to metamorphose the larvæ by injections of iodized proteins but his animals died. He concluded that such iodized proteins are highly toxic for thyroidless animals but not for those possessing normal glands. In my own experiments the axolotls withstood the injections of iodized amino-acid and serumglobulin as well as those with pieces of the gland present. There was nothing in the behavior of the animals to indicate that iodized substances are more toxic for thyroidless axolotls than for normal animals. Iodized substances are certainly not more toxic for thyroidless anuran tadpoles than for normal larvæ.

In an earlier experiment than those recorded here three axolotls were thyroidectomized and injected with large doses of iodotyrosine. The animals were kept in ordinary glass aquaria without running water. All of the animals died within ten days following injection. In later experiments the injected axolotls were kept in large concrete tanks, filled to capacity and with fresh water running constantly. None of the animals died. Intraperitoneal injections of large doses of either iodotyrosine or iodoserumglobulin has a marked depressing effect upon thyroidless axolotls; the animals are sluggish, move about very little, refuse food, and show indications of weakness for several days or even a week following injection.[2]

[2] A large axolotl was thyroidectomized and kept for eight months then twice injected at eight day intervals with large amounts of iodized casein. Metamorphosis resulted within twenty-nine days.

The experiment shows beyond doubt that thyroidless urodele larvæ respond to iodized substances in precisely the same manner as thyroidless and pituitaryless anurans. The importance of iodine in the amino-acid and protein molecule in order to render these substances effective in inducing metamorphosis is clearly demonstrated.

II. Experiments on Larval *Spelerpes bislineatus*.

H. H. Wilder years ago ('99) called attention to *Spelerpes* as a favorable object for experimentation. Unlike most urodeles, the larvæ of *Spelerpes* can be obtained at any season of the year and are easily kept under laboratory conditions. The larval life of this form has not been adequately investigated, consequently little is known concerning it. I. W. Wilder ('22) has been engaged for some years in studying the relation of growth to metamorphosis but to date has published only a very brief summary of the results. The data indicate a large range of variation in the size and age of the animals at transformation, with an average larval life of two years.

Eighty animals were used in the experiment, varying in size from 23 mm. to 53 mm. total length. They were separated into four groups of twenty animals each, equal numbers of large and small larvæ being represented. One group of twenty animals was kept in a large aquarium and given plenty of food. The three remaining cultures were subdivided into smaller lots of ten animals each and kept in glass containers in 250 cc. of tap water water through which compressed air bubbled constantly. *Spelerpes* larvæ soon die if kept in small amounts of water unless it is kept cool and well ærated. Twenty individuals were reared in tyrosine solutions representing 120 mg. per 250 cc. of water; another twenty were kept in equivalent solutions of 3-5 dibromtyrosine; twenty more in equal concentrations of 3-5 diiodotyrosine. The animals were fed sparingly during the experiment. Once each week they were removed from the tyrosine solutions and placed in large containers holding 6,000 cc. of water, plentifully supplied with food and allowed to feed for thirty-six hours after which they were returned to the solutions.

The experiment began October 17. Thirteen days from the date the animals were placed in the solutions, two large larvæ of the iodotyrosine culture showed marked gill and tail-fin reduction. None of the other animals of this or other cultures showed any change. However, by November 5, *i.e.*, twenty days from the first administration of iodotyrosine, all of the larvæ kept in solutions of this substance had metamorphosed. The external gills had disappeared, the gill clefts had closed and the tail fin was completely resorbed. The animals left the water and crawled up the sides of the container.

Examination of the tyrosine and dibromtyrosine cultures showed no indications of metamorphosis. The animals of these cultures were kept in the solutions for a month longer but metamorphosis was not induced. At the close of the experiment exceedingly strong concentrations of tyrosine and dibromtyrosine were employed but with negative results. The larvæ of the normal culture kept in the large aquarium and plentifully supplied with food likewise failed to metamorphose or to show any indications of transformation a month after the animals of the iodotyrosine culture had completed the process.

The results of this experiment are quite clean cut and admit of but one interpretation: It is the iodine within the tyrosine molecule that is responsible for the induced metamorphosis because the tyrosine and dibromtyrosine were ineffective either in weak or strong concentrations when administered over comparatively long periods.

Efforts were made to thyroidectomize the larvæ but without success owing to the extremely small size of the thyroid glands. The thyroid apparatus of *Spelerpes* larvæ of 47 mm. total length does not contain enough of the physiologically active hormone to induce metamorphosis in anuran tadpoles when heteroplastically transplanted. This experiment was attempted by Mr. O. M. Helff of this laboratory but without success.

Judging by the positive results obtained with thyroidless axolotls, it is highly probable that thyroidless *Spelerpes* would react by rapid metamorphosis if injected with, or reared in solutions of iodotyrosine. It is interesting to note that both large and small larvæ metamorphosed within twenty days, though the differences in size were great, varying as they did from 23 mm. to 52 mm. total length. Probably some of the smaller animals were considerably younger than the larger ones, however, it is impossible to make any definite statements on the point until more is known about the relation of size to age in the larvæ of this urodele.

The fact is well known that the thyroid gland of vertebrates exhibits a remarkable selective action in regard to iodine absorption, taking this element from the blood and synthesizing it into the thyroid hormone by the addition of other substances. In view of this property of thyroid tissue, it is possible, though rather improbable, that in the experiment just cited, the thyroid apparatus of the iodotyrosine-fed animals took up the iodine pouring into the organism and elaborated excessive quantities of the hormone, thus inducing metamorphosis. However, the rapid transformation of both thyroidless frog and salamander larvæ when fed or injected with iodized proteins and amino acids renders such an assumption doubtful.

It is interesting to note that Huxley and Hogben metamorphosed *Salamandra* and *Triton* larvæ by rearing them in dilute solutions of inorganic iodine. But in these experiments the thyroid glands of the animals were intact, hence it is impossible to know whether the action of the iodine was direct or through the mediation of the thyroid.

III. EXPERIMENTS ON *Amblystoma punctatum*.

It was considered desirable to test the effects of iodo- and bromtyrosine upon the metamorphosis of *Amblystoma* since it was owing to negative results obtained by administration of inorganic iodine to animals of this group that led Uhlenhuth to assert that iodine has no effect upon salamander transformation.

Eighty young larvæ of *Amblystoma punctatum*, averaging 30 mm. total length were divided into four groups of twenty animals each. The larvæ of three groups were isolated in finger bowls containing 50 c.c of water, one animal to each container. The twenty larvæ remaining were reared in large aquaria and fed quantities of tubifex. The animals in the finger bowls received food only at definite intervals and in very small amounts so that they were in a state of semi-starvation during the course of the experiment.

Twenty larvæ received small amounts of diodotyrosine crystals dissolved in the 50 cc. of water in the finger bowls. Twenty larvæ were fed equal quantities of dibromtyrosine crystals, whereas the remaining twenty animals received no food of any kind and served as controls. Such a culture was considered necessary in order to note any effects starvation might bring about on the progress of metamorphosis since the animals reared on the tyrosine compounds were given very little food.

It should be pointed out here that the animals in the dibromtyrosine solutions received considerably more bromine than the animals of the iodotyrosine cultures received iodine, despite the fact that equal quantities of the two substances were fed to each larva. This is obvious enough since the atomic weight of iodine is 126.92, while that of bromine is but 79.92. Consequently if equal amounts of the two tyrosine compounds were fed or put into solution in given amounts of water as was the case in the present experiment, the number of bromine atoms per milligram of dibromtyrosine would be nearly double the number of iodine atoms per milligram of diiodotyrosine. In all the experiments recorded in this paper this fact has been ignored and the two tyrosine compounds have been administered in equal amounts as though they were chemically equivalent. The experiment began June 22, 1923. On this date none of the small immature larvæ revealed the slightest indication of metamorphosis. June 28 all animals of the iodotyrosine-fed culture showed marked reduction of the gills—three animals had only the stumps remaining. The tail-fin was undergoing reduction. Sand was placed in each finger bowl in order that the animals might crawl out of the water as metamorphosis progressed; this is a necessary precaution, otherwise the animals will drown over night.

Examination of the larvæ reared in the dibromtyrosine solution showed no change; this was also true of the fed and unfed control cultures.

July 4, twelve days from the beginning of the experiment, all of the diiodotyrosine-fed larvæ had completely transformed and left the water. Their gills and tail-fin had disappeared; the branchial clefts had closed and the larvæ had shed their skins but the pigmentation characteristic of the adults of this species had not appeared. Fig. 1, *A*, is a photograph of four iodotyrosine-fed animals. Compare this with Fig. 1, *B*, which

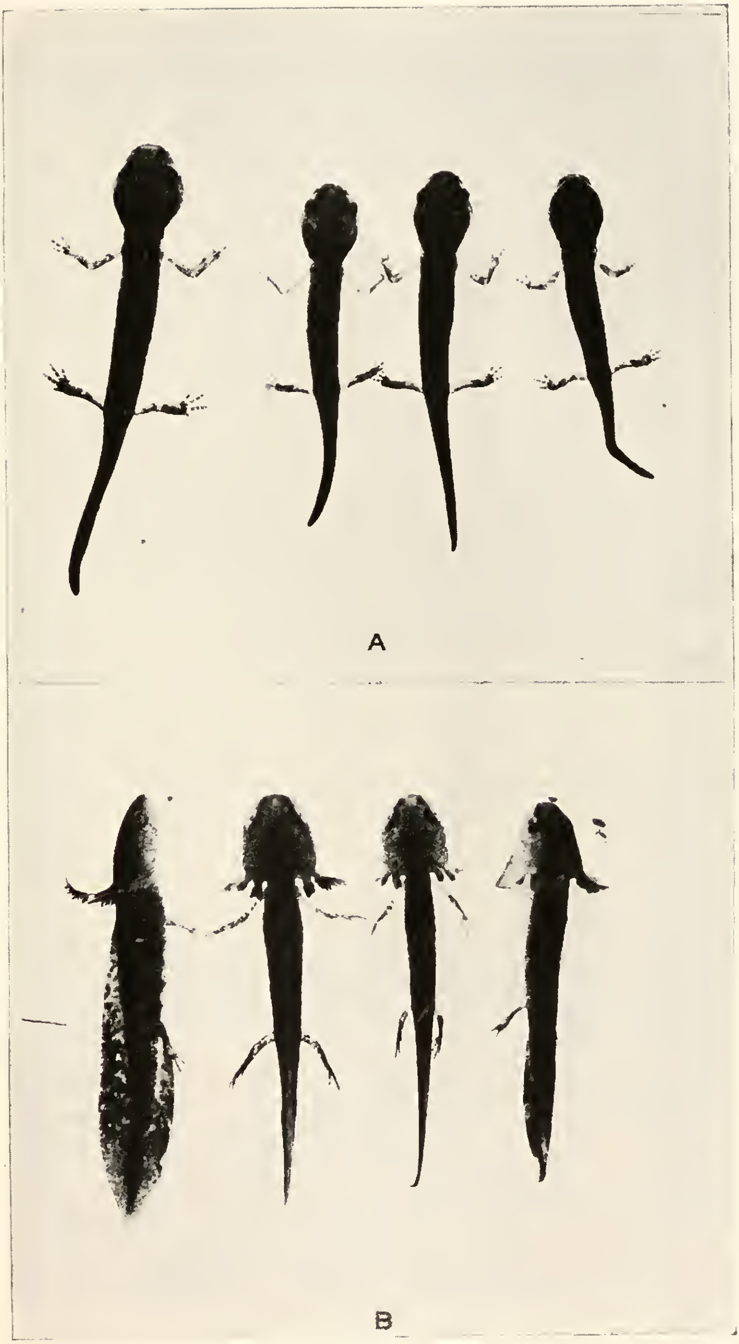

Fig. 1. *A*. Precocious metamorphosis of immature *Amblystoma* larvæ after twelve days immersion in iodotyrosine solution. × 2. *B*. Immature larvæ of *Amblystoma* after twenty-five days immersion in dibromtyrosine solution. × 2.

shows four di-bromtyrosine-fed larvæ. The animals in Fig. 1, *B*, were photographed twenty-five days after the experiment began, hence had been on the dibromtyrosine diet twice as long as the animals of Fig. 1, *A*, had been fed iodotyrosine. The metamorphosed animals were very weak and died within three to five days after metamorphosis. During the twelve days the experiment continued, they were each fed three small worms, the animals of the other cultures (except one) received the same.

The dibromtyrosine culture was continued twenty-six days after transformation of the iodotyrosine culture had occurred, *i.e.*, thirty-eight days from the date of first feeding. At the end of this time eleven animals metamorphosed, the remaining larvæ did not transform. The culture was abandoned July 31. The fed and unfed control groups were also given up at this time.

It is an interesting fact that none of the control animals of either starved or fed cultures metamorphosed during the thirty-eight days of the experiment, whereas eleven larvæ of the dibromtyrosine culture did transform. The experiment indicates that unfed salamander larvæ can be forced to metamorphose if reared in very strong solutions of dibromtyrosine over long periods. The bromine in the tyrosine molecule is thus seen to be not entirely inert as regards metamorphosis though it cannot be compared with iodine. For instance, diiodotyrosine solutions of about one-half the concentration of the dibromtyrosine, metamorphosed salamander larvæ of equal size and developmental stage in a period ranging from seven to twelve days, whereas the much stronger bromtyrosine solutions caused about two-thirds of the larvæ of the culture to transform between the thirtieth and thirty-ninth day.

In an earlier paper ('22) the writer called attention to the fact that the hind legs of thyroidless and pituitaryless tadpoles reared in strong dibromtyrosine solutions grow larger than the limbs of like animals on an algæ diet but that such animals do not metamorphose. Here again the evidence indicates that the bromine ion is not entirely passive since it does stimulate to a slight degree the growth of the tadpole's hind limbs. But the degree of activity of the bromine in the tyrosine molecule is not comparable to that of iodine. The following experiment clearly shows the great difference in activity between the two substances. Three sets of

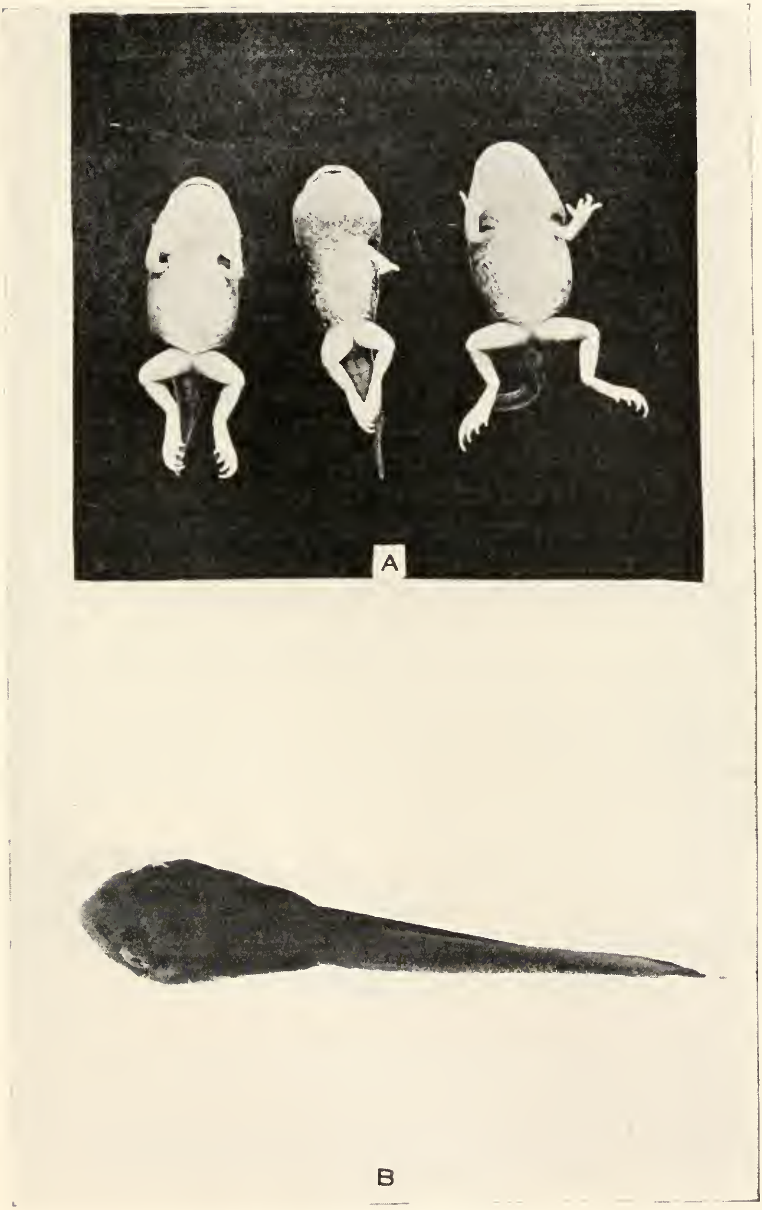

Fig. 2. *A*. Immature *Rana clamitans* larva metamorphosed by immersion in iodotyrosine solution for fifteen days. *B*. *Rana clamitans* tadpole reared for twenty days in dibromtyrosine solution.

thyroidless and pituitaryless *R. sylvatica* larvæ were used. One set of animals was reared in tyrosine solution, another in iodotyrosine, a third set in dibromtyrosine of twice the strength of the iodotyrosine solution. No food of any kind was given. The iodotyrosine cluture metamorphosed within fifteen days, the animals of tyrosine and dibromtyrosine culture showed no indications of metamorphosis after forty-three days. However, the larvæ reared in the dibromtyrosine solutions had well developed hind legs averaging 7 mm. total length as compared with an average of 3.5 for the animals of the tyrosine culture. Since no food of any kind was given the increase in limb growth was probably due to a slight influence exerted by the bromine ion.

The result of this experiment upon *Amblystoma punctatum* larvæ is identical with that obtained with *Spelerpes*, and shows clearly that it is the iodine in the tyrosine molecule that is responsible for the precocious metamorphosis. The rapidity with which the metamorphic response to iodotyrosine is evoked in salamander larvæ, would seem to indicate that the action of the iodized amino-acid is directly upon the cells and tissues of the organism and not necessarily through the intermediation of the thyroid gland. It will be recalled, that young thyroidless axolotls (*Amblystoma tigrinum*) transform following injections of iodotyrosine and it is probable that thyroidless *Amblystoma punctatum* would react likewise. However, no experiments were made to test this point.

The writer now has under way a series of experiments upon the metamorphosis of thyroidectomized salamander and anuran larvæ in which various iodized proteins and amino acids are fed and injected. The results will be communicated later.

IV. Experiments on *Rana clamitans* Tadpoles.

The larval life of the green frog, *Rana clamitans*, extends over a year (370–400 days according to Wright, '14) and in some individuals is prolonged two years before metamorphosis occurs. This form offers exceptional opportunities for experimentation because of the long duration of larval existence, and is especially valuable for use in investigations on metamorphosis where the feeding method is employed. Tadpoles of all sizes are obtainable at any season of the year and when brought to the laboratory readily adapt themselves to the changed environment. Animals captured in the autumn generally pass the winter and spring as tadpoles.

September 13, 1922, a large number of larvæ were collected and eighty tadpoles of approximately equal size and developmental stage were selected and divided into four groups of twenty individuals each. One group was fed tyrosine, another ordinary tadpole food such as spirogyra and insect larvæ, the third lot received dibromtyrosine and the fourth group diiodotyrosine. A fifth culture of animals, forty in number, of varying size and developmental stage was kept in large glass aquaria and fed quantities of algæ. Each culture except the fifth was further subdivided into lots of five tadpoles each, and placed in 250 cc. of tap water containing 120 mg. of the tyrosine compounds. Each evening the animals were transferred to large jars containing 10,000 cc. of tap water and plentifully supplied with algæ. This procedure was considered necessary in order to rule out the starvation factor since tadpoles can not exist indefinitely on tyrosine alone.

The animals obtained the tyrosine compounds by two methods: through the alimentary tract, and by absorption through the skin. Most of the tyrosine goes into solution after a short time, hence the larvæ probably obtain most of this substance through the skin. This is certainly the manner in which *Spelerpes* larvæ obtain the tyrosine and its iodized and brominated compounds.

Table I. gives the average measurements of the animals at the beginning and end of the experiment. The figures are averages based upon measurements of fifteen animals of each culture.

TABLE I.

	Algæ-Fed.		Tyrosine.		Dibromtyrosine.		Diiodotyrosine.	
	Total Length, mm.	Hind Legs, mm.	Total Length, mm.	Hind Legs, mm.	Total Length, mm.	Hind Legs. mm.	Total Length, mm.	Hind Legs. mm.
Sept. 13.....	51.2	3.9	52	3.5	52.2	3.8	51.5	3.5
Oct. 8.......	52.2	3.9	52.5	3.8	52.5	3.9	Advanced stages of metamorphosis	

Thirteen days from the beginning of the experiment a marked difference was observed between the iodotyrosine-fed animals and those of the other cultures. The tyrosine and dibromtyrosine-fed tadpoles showed no changes either in regard to growth or metamorphosis from the algæ-fed controls, whereas the iodotyrosine-fed animals appeared thin and emaciated; the hind legs had increased in length also the skin of the pectoral region where later the fore-legs appear, was undergoing autolysis.

October 3, four of the iodotyrosine-fed tadpoles had fore legs and frog mouths. The hind legs of all of the animals had markedly increased in length, and the tail was undergoing resorption. In several individuals large patches of skin over the region of the fore-limbs was totally destroyed by autolysis. The animals of the remaining cultures showed no change.

October 6, the animals of the iodotyrosine culture were all in advanced stages of metamorphosis and many were dying; only one animal remained alive on October 8. Table I. gives the measurements of the tadpoles of the various cultures at the close of the diiodotyrosine experiment, twenty-three days after the date of first feeding.

The remaining cultures were continued until October 30. The tyrosine and dibromtyrosine-fed tadpoles were then taken off the tyrosine diet and fed algæ. The larvæ were measured November 13 two months after the beginning of the experiment but no indications of metamorphic change was observed. It is quite evident that insofar as the metamorphosis of the green frog is concerned, tyrosine and dibromtyrosine are ineffective, even when administered in large quantities. These results are in agreement with those obtained in similar experiments on thyroidless axolotls and thyroidless and pituitaryless anuran tadpoles (Swingle, '22).

Discussion.

The experiments described in this paper and elsewhere on the administration of iodine, iodized amino-acids and proteins to thyroidless and pituitaryless anuran larvæ and to thyroidless axolotls demonstrate that other forms of iodine than that peculiar to the thyroid hormone (thyroid iodine) possess the power of inducing amphibian transformation. This property of iodine is apparently unique, since so far as known at present it is not shared by other substances, and is inherent in the iodine atom when organically combined in a certain way. The type of combination is not necessarily that characteristic of thyroid iodine because a large number of iodine compounds have the power to bring on metamorphosis in thyroidless amphibians—even elemental iodine itself. That elemental iodine is utilized within the organism as iodine per se for any purpose seems improbable because if one compares the effect of administering inorganic iodine and various organic preparations to thyroidless tadpoles, it becomes evident that the physiologic activity and metamorphosis-inducing properties of the organic preparations are superior to elemental iodine. Furthemore, the accelerating effect upon metamorphosis of the organic iodine compounds is less than that of the thyroid extract itself. However, the important thing is not the speed with which iodine compounds induce metamorphosis in comparison with the thyroid hormone itself, but the fact that iodine other than thyroid iodine induces the metamorphosis of thyroidless animals. The crux of the problem in regard to amphibian metamorphosis, is to find out just what it is in tyrosine, serumalbumen, serumglobulin, casein, tyramine and probably a host of other amino acids and proteins which when iodine is added increases so remarkably the metamorphosis-inducing powers of this element. Why, for example, does the linking of iodine to the third and fifth carbon atoms of the benzene ring in the tyrosine molecule transform this inert (so far as metamorphosis is concerned) amino acid into a highly active agent. What is it in casein, albumen, globulin or tyrosine that raises so greatly the reactive powers of iodine?

There can be little doubt that elemental iodine when it induces the transformation of thyroidless and pituitaryless tadpoles combines either with the proteins of the algæ fed along with it, or within the body of the tadpole after absorption through the skin or alimentary tract, but it doesn't combine with anything produced by thyroid tissue because there was none present nor had there ever been any present in the case of the thyroidless anurans.

Hirschler ('22) precociously metamorphosed tadpoles by inserting small pieces of elemental iodine into the body of the larvæ. However, since the animals possessed intact thyroid glands their activity can not be ruled out in this experiment, because the metamorphosis may have been due to increased activity of the thyroid due to increased iodine supply. On the other hand the absorbed iodine may have combined with other than thyroid proteins thus inducing metamorphosis. Either or both of these possibilities may have been realized in Hirschler's experiment, consequently work involving iodine administration should be performed only on thyroidectomized larvæ since only this type of experiment will shed any light upon the rôle of iodine in amphibian metamorphosis. This is especially true of neotenous forms like axolotl; no work on the rôle of iodine in the metamorphosis of this form should be regarded as conclusive unless performed upon animals from which all trace of thryoid tissue has been removed.

Thyroid conditions in axolotl are very peculiar (Swingle, '22) and vitiate the results of experiments done on animals with intact glands. The New Mexican strain of axolotl has perfectly developed thyroids, the vesicles filled to capacity with the physiologically active hormone yet the secretion is apparently unable to escape into the blood stream in sufficient quantities to transform the animal. This is demonstrated by heteroplastic thyroid transplants. An axolotl thyroid is sufficient to metamorphose thirteen normal, thyroidless and pituitaryless anuran tadpoles when grafted, but left intact within the axolot's body is incapable of initiating metamorphosis.[1] It is obvious that in this form we are dealing with a thyroid mechanism which selects, stores and transforms the iodine of the animal's food and water into the thyroid hormone but fails to release the elaborated product. Consequently, if axolotls are fed elemental iodine they do not metamorphose, and for exactly the same reason, they fail to transform under the ordinary dietary regime—the iodine is picked up by the gland and synthesized but not released. It is probable that feeding organic iodine preparations would give similar results. On the other hand it is erroneous to conclude from such an experiment that iodine has no influence on axolotl metamorphosis. Our experiments on both thyroidectomized and partially thyroidectomized axolotls show quite clearly that iodized amino acids and proteins promptly metamorphose these animals, and that the amino-acid and protein without the iodine in the molecule are inert.

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[1] This experiment was performed by Mr. Karl Mason, of this laboratory, and reported at the Boston meeting of the American Society of Zoölogists, 1922.

Hirschler ('22) metamorphosed axolotls (the European strain which rarely spontaneously transforms) by implanting iodoform paste within the body cavity. However, in this experiment the glands were intact so it may be that the iodoform merely served to stimulate the thyroid mechanism to release its stored hormone thus inducing metamorphosis. It will be recalled that Kaufman ('18) metamorphosed axolotl by injections of salicylic acid. So far as is known salicylic acid has no influence on metamorphosis, the effect of injecting the substance into axolotl was to stimulate the secretory activity of the thyroid apparatus in some way. Anyone not familiar with thyroid conditions in axolotl might conclude that salicylic acid per se was the metamorphosis-inducing agent, whereas probably nothing could be farther from the real facts of the case.

The New Mexican strain of axolotl if removed from its native habitat to New Haven soon undergoes spontaneous metamorphosis. Why? Certainly not because the railroad journey exerts any mysterious metamorphosis-inducing power, but probably because the changed food, water, the jolting and confinement incident to the trip acted as a stimulating agent thus releasing the thyroid hormone from the gland vesicles thereby causing transformation.

Where the thyroid apparatus is left intact the fate of the substances fed or injected into an amphibian larva is problematical. If an effect upon metamorphosis is produced it is impossible to determine whether the effect is due directly to the substance itself, or indirectly through intermediation of the thyroid unless the work is checked by repeating it upon thyroidless forms. An excellent illustration of this statement has been furnished by Allen ('20). Allen observed that transplantation of the anterior lobe of the pituitary into hypophysectomized tadpoles (but possessing a rudimentary and functionless thyroid apparatus) metamorphosed the animals, whereas transplantation of the pituitary gland into thyroidectomized larvæ had no such effect. The pituitary secretion had no direct influence upon metamorphosis but acted indirectly by stimulating the rudimentary thyroid into functional activity. Any one working with normal tadpoles with intact thyroids uncontrolled by thyroidless animals might well have concluded from the results of such an experiment that pituitary tissue exerts a direct stimulus to metamorphosis and is thus equivalent to the thyroid.

Recently ('22) Romeis claims to have isolated an absolutely iodine-free substance from the thyroid gland which when administered to frog tadpoles exhibits all of the physiologic effects of thyroid gland tissue. The writer is skeptical of the validity of this claim and for several reasons: (1) All active substances so far isolated from the thyroid contain large amounts of iodine. Thyroxin the active principle contains sixty-five per cent. of iodine as an integral part of the molecule; and it is known that thyreoglobulin containing no iodine is physiologically inert; (2) Experiments on tadpoles have shown (Rogoff, '18–19) that blood coming from hyperplastic thyroid glands with extremely low iodine content fails to induce tadpole metamorphosis; (3) To date the only substances known to metamorphose thyroidless tadpoles are thyroid or iodine in some form—presumably in the last analysis organically combined. While writing this paper the writer came across a second communication from Romeis which practically amounts to a retraction of his earlier claims. He found that iodothyrine and iodothyroglobulin will induce the metamorphosis of tadpoles in dilutions of one in a million; thyroxin produced the same effects on growth and metamorphosis in dilutions of one in ten million consequently says Romeis: These figures suggest that effects (on tadpoles) from so-called iodine-free materials from the thyroid may have been contaminated with minute amounts of thyroxin.

There can be little doubt that this is the real explanation of the results obtained by this investigator with so-called iodine-free substances from the thyroid.

There seems to exist a fundamental difference between mammals and amphibians in regard to their physiologic response to thyroid and iodine administration. This difference is not sufficiently understood by those who attempt to compare these two verebrate groups. The following experiment of Kendall ('19) is an excellent illustration of the point: He found that injections of pure thyroxin into mammals is followed by a very definite and marked physiologic response. But when the hydrogen of the imino group in the thyroxin is replaced with acetyl, the substance loses its physiologic activity and there follows no demonstrable effect upon the metabolic rate. This emphasized the importance of the imino group in thyroxin (in so far as the metabolic effect upon mammals is concerned) and minimizes the importance of the iodine in the molecule. Bearing in mind the effect of iodine administration upon tadpole metamorphosis, Kendall was led to try the acetyl derivative of thyroxin on tadpoles, for if metamorphosis depends only upon the increase in the basal metabolic rate of the larvæ then thyroxin should increase the rate of metamorphosis but the acetyl derivative involving the imino group should not. If, however, iodine alone is concerned in accelerating metamorphosis, then both thyroxin and the derivative should affect the transformation. Kendall found that both thyroxin and the acetyl derivative would induce a rapid metamorphosis of the bull frog tadpole. Kendall's conclusion was that thyroxin appears to have two separate and distinct functions: the effect upon the metabolic rate which is brought about by the CO-NH groups within the molecule; and the physiological changes involved in the metamorphosis of the tadpole due to the iodine contained in the molecule. Our own experiments have demonstrated that this action of iodine is not specific to thyroxin, but can be obtained in thyroidless amphibians (though the effects are not so rapid, and the amounts administered must be larger) by a large number of other iodine compounds and by administration of elemental iodine itself.

Kendall's experiment sheds considerable light on the reason for the conflicting results obtained by investigators working with iodine, iodized proteins and amino acids on mammals, and the students of amphibian metamorphosis. In mammals the criterion employed for testing the phsiologic action of iodine and thyroid upon the organism is the effect upon metabolism as indicated by changes in the nitrogen excretion, CO_2 elimination and oxygen consumption; in amphibians the criterion has been the rate and degree of the degenerative and regenerative processes incident to metamorphosis. However, it is becoming clear that the two types of physiologic response are not in the same class and hence not to be compared because they owe their origin to different causes. The unique effects of thyroid or thyroxin upon the metabolic rate is due to the specific chemical structure of the thyroxin molecule particularly the CO–NH group, whereas metamorphic response of amphibians is dependent upon a peculiar property of iodine in certain types of combination, although not necessarily that characteristic of thyroid iodine, *e.g.*, iodized amino acids and proteins. Further evidence of the difference as to cause between metabolic changes in mammals and amphibian metamorphosis is furnished by the acetonitrile test where iodized substances shown to be specific in accelerating metamorphosis completely fail to simulate the thyroid function in protecting mice against the lethal effects of actonitrile.

Hunt and Seidell ('09) made the interesting observation that feeding thyroid tissue to white mice greatly increases the resistance of these animals to lethal doses of acetonitrile, and that the efficiency of the gland seems dependent upon its iodine content. They concluded that the increased resistance of the mice to the poison was due to the changed metabolism of the animals following thyroid feeding, the metabolic change preventing the acetonitrile from breaking down into its posionous product hydrocyanic acid. This assumption was based upon the fact that thyroid feeding does not raise the resistance of mice to lethal doses of hydrocyanic acid itself.

Koch ('13) and Miura ('22) found that iodized amino acids such as diiodotyrosine iodotryptophan and tetra-iodohistidine when administered to mice fails to increase their resistance to acetonitrile.

Strouse and Voegtlin ('09–'10) failed to observe any thyroid-like effect on the nitrogen metabolism or on the blood pressure of normal dogs, nor was there any favorable effect on the condition of myxedematous and cretinous mammals following administration of iodized amino acid. Other investigators have tried in vain to obtain thyroid effects on mammals (metabolic changes) by the use of various iodized substances; tri-iodo-imidazol and, iodophenylalanie have also proven ineffective.

If, however, we bear in mind the results of Kendall's experiment, it becomes clear why iodized amino acids, *e.g.*, iodotyrosine, give negative results when administered to myxydematous and cretinous mammals, and positive results when fed to thyroidless amphibian larvæ. In the latter group the metamorphic response is due to the iodine in the molecule, in the former group the metabolic response depends upon something else, *i.e.*, the CO–NH group within the thyroxin molecule. This brings us to the consideration of another point, *i.e.*, the possibility of substituting other halogens for iodine and obtaining the same effects upon metamorphosis.

Kendall ('18) as a result of his investigations of the unique effects of thyroxin upon the metabolic rate of mammals, was led to conclude that insofar as the physiologic effect upon mammals is concerned, possibly other halogens could be substituted for the iodine of the thyroxin molecule without greatly changing the physiological properties of the thyroxin. His conclusion follows: "In regard to the relation of iodine to the activity of thyroxin, the presence of iodine in the compound must exert some influence, and it seems not improbable that the presence of iodine renders the active groups more reactive. In the absence of iodine it would take a greater working pressure to bring about its reaction. The substitution of iodine by hydrogen or chlorine or bromine would undoubtedly be followed by an alteration in the degree of reactivity of the substance but its gross chemical nature and properites would not be altered thereby."

Whether or not other halogens can or cannot be substituted for the iodine of the thyroxin molecule and this substance still retain its physiological activity in mammals is an open question, at any rate there are no experimental data tending to answer the question one way or the other except possibly the work of Ostwald and von Cyon who observed that thyreoglobulin containing no iodine was physiologically inert, whereas this substance gives all the physiological effects of thyroid tissue when iodine is present. However, this may be, it is clear that insofar as amphibian metamorphosis is concerned other halogens such as bromine can not be substituted for iodine. The experiments upon thyroidless axolotls and anuran larvæ where 3-5 dibromtyrosine was employed, demonstrates the futility of endeavoring to substitute bromine for iodine with hope of affecting metamorphosis. It is merely quibbling to say that bromine would probably be just as effective as iodine providing the organism possessed a mechanism for utilizing this halogen in the way the thyroid utilizes iodine to elaborate its hormone, because in our experiments the animals fed iodotyrosine and iodoserumglobulin had no vestige of thyroid tissue present, consequently had no mechanism for iodine utilization.

No one has ever shown that in the absence of thyroid glands, other tissues of the organisms have the power of functioning vicariously for the thyroid, and synthesizing its active hormone. If this possibility were true then why is it that mammals with atrophied or degenerate thyroids are quite unable to utilize iodine or iodized proteins and amino-acids. If other tissues of vertebrates besides the thyroid glands possess the power to manufacture the thyroid hormone it is strange that this power should be present in amphibia yet lacking in mammals. The truth of the matter is that amphibian metamorphosis depends upon a peculiar property inherent in the iodine atom when combined in certain ways.

Some investigators have claimed that the pituitary can function vicariously for the thyroid when the latter is absent, hence it might be said that in thyroidless amphibians the pituitary gland may synthesize the iodine into the chemical complex characteristic of the thyroid hormone. Aside from the total lack of evidence that the pituitary can function vicariously for the thyroid in thyroidless forms, the experiments of Allen ('19) are of interest in this connection. Allen extirpated both the thyroid and the pituitary gland of frog embryos and later fed the tadpoles with starch iodide. The animals underwent a precocious and nearly complete metamorphosis before death ensued, clearly demonstrating that iodine is as effective in inducing transformation in tadpoles lacking both thyroid and pituitary as in larvæ with only the thyroid missing.

Summary of Conclusions

1. Thyroidectomized, partially thyroidectomized, and normal axolotls are readily metamorphosed by intraperitoneal injections of iodotyrosine and iodoserumglobulin and iodocasein.

2. Thyroidectomized, partially thyroidectomized and normal axolotls do not metamorphose when injected with large quantities of pure tyrosine, 3-5 dibromtyrosine (two atoms of bromine in the molecule) and non-iodized serumglobulin.

3. Larval *Spelerpes bilineatus* readily metamorphoses in strong solutions of 3-5 diiodotyrosine, but do not transform in equivalent concentrations of tyrosine and 3-5 dibromtyrosine even when kept in such solutions over comparatively long periods.

4. *Rana clamitans* tadpoles with from six to eight months of larval life remaining (*i.e.*, passing the winter as tadpoles) were metamorphosed within twenty days by rearing the animals in strong concentrations of 3-5 diiodotyrosine and feeding them with this substance. Control larvæ of similar age and developmental stage reared in equivalent solutions of 3-5 dibromtyrosine and fed quantities of the compound failed to transform.

5. The experiments are clean cut and admit of but one interpretation; it is the iodine in the amino acid and protein molecule that is responsible for amphibian metamorphosis. Why the linking of iodine to the third and fifth carbon atoms of the benzene ring of the tyrosine molecule should so greatly increase the reactive powers of the iodine is unknown.

6. Iodine other than thyroid iodine is effective in inducing the metamorphosis of thyroidless urodele and anuran larvæ. There is no evidence that any other tissue of the vertebrate organism has the power to function vicariously for the thyroid in the latter's absence.

7. Bromine has little or no influence upon amphibian metamorphosis and can not be substituted for iodine. The substitution of two bromine atoms for two hydrogen atoms (the third and fifth) of the tyrosine molecule fails to change this substance into a metamorphosis-inducing agent.

8. The physiologic responses (metabolic changes) of mammals to thyroid administration are due to the CO–NH group within the thyroxin molecule; amphibian metamorphosis to the iodine in the molecule. Consequently thyroidless animals of these two vertebrate groups are hardly comparable in regard to their response to iodine. Only thyroid iodine containing the CO–NH group is effective in thyroidless mammals whereas iodine other than thyroid iodine will metamorphose thyroidless amphibian larvæ.

9. Data are presented showing why experiments on amphibian larvæ devised to test out the effect upon metamorphosis by feeding or injecting various substances are unsound unless per-

formed upon thyroidless animals or at any rate controlled by identical experiments upon thyroidectomized individuals.

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SOME NOTES ON THE FERTILIZATION REACTION IN ECHINODERM EGGS.

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The following observations make no claim to novelty but are perhaps worthy of record as additional evidence on certain matters. They are concerned solely with the fertilization reaction, that is to say, with the activation of the egg, and not with cleavage. Activation and cleavage must, I think, be regarded as distinct processes.

The observations were made in May, 1922, at the Hopkins Marine Station, Pacific Grove, California. I am indebted to the director, Dr. W. K. Fisher, for the privilege of working at the station and for his unfailing promptness in supplying me with everything necessary for my work.

1. Materials and Methods.—The eggs used were those of the Pacific coast sea-urchins, *Strongylocentrotus franciscanus* and *purpuratus*, and of the starfish, *Patiria miniata*. The latter is the *Asterina* of Loeb. The eggs of the urchins were obtained by removing the oral portion of the test, leaving the ovaries in the aboral portion. All other viscera and tissues were then removed from the latter and it was rinsed several times with sea-water. Upon standing for a short time, those portions of the ovaries which are ripe break down, releasing the eggs, which may then be removed with a pipette. The urchins were evidently past their prime at the time during which I worked with them as only small portions of the ovaries contained mature eggs. These seemed, however, to be entirely normal in most cases, and gave a high percentage of fertilization membranes and cleavage.

The eggs of *Patiria* employed were normally shed eggs. It was found that when these starfish are spread out on a table, a considerable number of them (presumably those that happen to be ripe) will begin to shed eggs and sperm and continue this for three or four hours. It may be noted in passing that the normally shed eggs of *Patiria* are fully mature, are immediately fertilizable as they exude from the genital pores, and yield 100 per cent. fertilization membranes, cleavage, and larvæ. This agrees with observations on other starfish. As is well known, and this is also the case in *Patiria*, starfish eggs obtained by shaking or mincing the ovaries are not mature, but must stand in sea-water for some time before they attain a fertilizable condition.

In the following account a number of statements are made concerning viscosity differences. These have been determined in the following manner. The eggs are placed on a slide in a drop of sea-water without cover. Low power was used (Leitz objective 2, ocular 3). Any egg to be investigated was rapidly pushed with a needle to the periphery of the drop and then into a small evagination of the periphery where it is held by surface tension. It was then punctured or cut with a needle and the rate and readiness with which the egg cytoplasm flows out under the pressure of surface tension as well as the duration of retention of cuts and gashes furnish a relative measure of the viscosity of the cytoplasm. The needle used was a fine steel needle thrust into a wooden handle and operated by hand. The method is somewhat crude but has the advantages that it is rapid and almost entirely objective.

2. *Viscosity of the Unfertilized Mature Egg.*—The unfertilized egg in all three species consists of a slightly viscous cytoplasm inclosed in a definite membrane of considerably greater consistency than the cytoplasm. This membrane, which may be designated the vitelline membrane (it has also been named plasma membrane and egg membrane) is probably a colloidal gel. It is more delicate in the sea-urchin than in the starfish egg.

These facts have been determined as follows. When the sea-urchin egg (either species) is held by surface tension and punctured with a needle, the cytoplasm rushes out with almost explosive force and the whole egg very rapidly disintegrates. In this disintegration the vitelline membrane is also involved so that I at first thought such a membrane was absent. However, if the eggs are rapidly pushed back into the drop when they are partially disintegrated, egg fragments of various sizes are obtained. On the surface of such fragments wrinkles are always observable. It is evident that such wrinkles must be located in a surface membrane of greater consistency than the cytoplasm, in fact, of solid consistency, as fluids do not exhibit permanent wrinkles. These wrinkles on egg fragments are illustrated in Figs. 10 and 13. In only one case did the entire membrane persist after disintegration of the cytoplasm; this case is illustrated in Fig. 16. The membrane is collapsed and wrinkled.

In the *Patiria* egg, when punctured as just described, the cytoplasm flows out invariably leaving the membrane behind. This is illustrated in Figs. 19 to 25. From the fact that the membrane always persists in the starfish egg after disruption of the cytoplasm and rarely so persists in the urchin egg, I have drawn the conclusion that the vitelline membrane of the former egg is firmer, tougher, and probably thicker than in the latter egg. This difference is further evidenced by the fact that the empty membrane in *Patiria* retains its former shape better than in the urchins as may be seen by comparing Figs. 16 and 19.

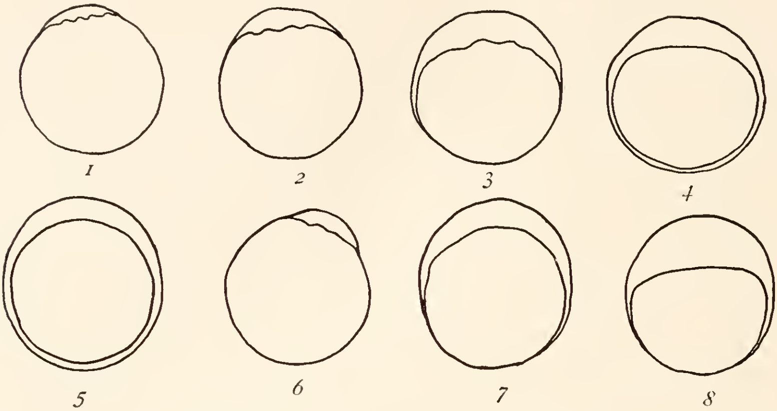
The consistency of unfertilized eggs has been previously described by a number of investigators. The first detailed description of the viscosity conditions in unfertilized eggs seems to have been that of Herbst ('93). Herbst noted that if pressure is applied to unfertilized sea-urchin eggs, the contents flow out and a fine membrane is sometimes left behind to which bits of protoplasm still cling. He therefore concluded that the surface layer of the egg possesses a greater consistency than the remainder of the egg but is not definitely separated from the latter. Lillie ('06) notes that the *Chætopterus* egg is semifluid and surrounded by a delicate membrane. Heilbrunn ('15) states that the cytoplasm of the unfertilized *Arbacia* egg is "typically fluid" and inclosed in a membrane described as being "a protein gel" and possessing "a certain degree of rigidity." Chambers ('17*a*) finds that the protoplasm of the unfertilized eggs of *Arbacia*, *Asterias*, *Echinarachnius*, *Cerebratulus*, and *Fucus* is a "hyaline fluid" of "very slight consistency" while the surface layer is "very dense in consistency as compared with the cell interior into which it merges insensibly." Chambers has also noted the greater delicacy of the membrane of the urchin egg than of the starfish egg ('21*b*). Heilbrunn ('20*b*) describes the *Cumingia* egg as "a mass of fluid protoplasm surrounded by a rigid membrane." Seifriz ('18, '20) finds that ripe *Fucus* eggs are decidedly viscous with a wall consisting of a very rigid hyaline gel; he also describes the cytoplasm of *Tripneustes* and *Echinarachnius* eggs as of about the consistency of glycerine. The cytoplasm of the eggs which I have investigated seems to me to correspond to 4 or 5 in Seifriz's scale ('20, p. 364). The existence of a membrane around the unfertilized egg has also been asserted by many other investigators apart from considerations of viscosity differences.

The vitelline membrane is probably not sharply delineated from the less viscous egg cytoplasm. According to Chambers ('17*a*) the external jellied surface of the egg cell "gradually passes into the sol of the interior." This gradation must be very abrupt as I did not notice it in my experiments although I think the superficial cytoplasm does undoubtedly adhere to the vitelline membrane.

3. The Morphology of the Fertilization Process.—The fertilization reaction has been observed on eggs mounted in plenty of water without a cover glass; on eggs in a depression slide with a cover glass; and on eggs in a hanging drop on the under surface of a cover glass placed over a depression. The reaction has been studied with low, medium, and high powers. The phenomena observed were the same by any method of mounting the eggs and cannot possibly be ascribed to compression, for the eggs were never under compression.

The fertilization reaction begins to be visible 45 to 60 seconds after mixing the eggs with dilute sperm suspension. It is first indicated by a roughening or crenation of the egg at one place on the surface and this roughening spreads very rapidly in all directions from the initial place over the entire surface of the egg. Following closely upon this change the vitelline membrane begins to elevate. This elevation starts at the same place as the roughening and like the latter sweeps in all directions over the egg. The roughening and the elevation of the membrane occur at such a close interval as to be almost simultaneous but it is certain that the former precedes. There is also to be noted a flattening of the egg at the region of initial membrane elevation. This flattening is commonly very marked at the site of initial membrane elevation and spreads from this for a short distance but never extends more than halfway over the egg. The fertilization changes in *S. franciscanus* are illustrated in Figs. 1 to 5.

The time required for these changes to pass over the egg is about 15 to 30 seconds in the best eggs. In subnormal eggs the time is much longer and the reaction may not be complete, the eggs remaining permanently with membranes partially elevated as shown in Figs. 6 to 8. Such cases serve, I think, to indicate further the progressive character of the fertilization reaction.



All figures are redrawn from free-hand sketches. In the unfertilized egg the vitelline membrane is not indicated separately from the egg surface as doing so would exaggerate the real appearance.

FIGS. 1 TO 5. Five stages in the normal fertilization reaction in *Strongylocentrotus franciscanus*. Note progress of the fertilization reaction from the initial place. In 5 the reaction is not yet quite complete as the egg is still slightly excentric within the membrane.

FIGS. 6 TO 8. Permanent stages of partial fertilization from a subnormal lot of eggs of *S. franciscanus*.

In eggs favorably placed for such observation it has been determined that the point on the egg from which the fertilization changes take their origin is the place to which the successful sperm is attached. This has also been ascertained by so many previous observers, to whom reference will be made shortly, that it seems superfluous to dwell upon the fact. The fertilization reaction begins at the point of contact of sperm and egg and from this point is transmitted in all directions over the surface of the egg.

The fertilization reaction is essentially the same in all three species studied. The chief difference between the urchin egg and the starfish egg is that in the former the membrane where it first separates from the egg elevates at once in this region to its fullest extent whereas in the starfish egg the membrane separates from the entire surface of the egg before it elevates to any considerable extent (Fig. 9). Consequently in the urchins the egg at first lies very asymmetrically placed within the membrane (Figs. 4 and 5). This asymmetry is further emphasized by the flattening of the egg at the region of initial elevation. Later symmetry is restored in normal eggs by the widening of the perivitelline space at other regions and by the resumption of spherical form by the egg. In subnormal eggs the asymmetry is likely to persist. In the *Patiria* egg, the membrane separates from the whole surface of the egg with the formation of only a very narrow perivitelline space; later this widens simultaneously around the egg. The flattening at the region of initial membrane elevation is slight in the starfish egg but generally perceptible.

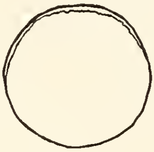


FIG. 9. Stage in the fertilization reaction of *Patiria miniata*. The vitelline membrane separates only slightly from the egg at first. Note also the crenation of the egg surface.

The separation of the vitelline membrane does not proceed with entire smoothness from the point of initiation but the membrane tends to adhere to the egg surface at some points slightly longer than at others. An exaggeration of this tendency results in the vesicle formation described and figured by Loeb ('13), observable according to him through retarding the normal reaction by lowering the temperature. Vesicle formation also occurs at ordinary temperatures in subnormal eggs and in such eggs the vesicles may persist, not flowing together to form a continuous perivitelline space. Pronounced vesicle formation seems to be due to a subnormal response of the egg to fertilization.

An attraction cone at the site of sperm entry such as has been reported by others was not noticed but was not particularly sought for. I was also unable to observe the passage of materials from the cortex into the perivitelline space, as described by Just ('19) for *Echinarachnius*, although I searched carefully for such a process.[1]

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[1] In making this statement I do not intend to imply the slightest question of the validity of Just's observations. I believe from other lines of evidence that material does pass from the egg into the perivitelline space but the process is invisible in the eggs with which I have worked.

The progressive character of the fertilization reaction has been previously described by a number of investigators. It was first noticed about simultaneously by Fol ('77, '79) and Calberla ('78). They observed that the detachment and elevation of the fertilization membrane are initiated at the point of attachment of the successful sperm and proceed from there in all directions around the egg. Fol[1] described this process for *Asterias glacialis*, *Toxopneustes lividus*, and *Sphærechinus brevispinosus*. Calberla's investigations concern the *Petromyzon* egg. This egg possesses a micropyle; no change occurs until the sperm has passed the length of the micropyle and touched the surface of the egg cytoplasm inside the membrane. When this happens the egg withdraws a little from the vitelline membrane at the micropyle and flattens slightly; the separation of the membrane from the egg followed by its elevation then proceeds from the micropyle over the egg. Théel ('92) describes the elevation of the membrane in the sea-urchin *Echinocyamus* in the following words: "At the place where the first sperm has penetrated the mucilaginous investment, a very thin plasmatic membrane rises and separates from the egg, beginning at the place of contact and extending eventually around the yolk." Herbst ('93) agrees with Fol's description of the fertilization reaction in the sea-urchin egg. Ries ('09*a*) also observed the progressive character of membrane elevation in the sea-urchin egg and presents photographs of the process, taken with a motion picture machine. Elder's drawing of the elevation of the fertilization membrane in *Strongylocentrotus purpuratus* shows that this process is a progressive change initiated at one place but Elder does not mention this in the text ('12). Okkelberg ('14) has described the elevation of the membrane in the egg of the brook lamprey *Entosphenus wilderi*. In this egg fertilization occurs at the animal pole and the membrane here separates from the egg. A wave of contraction then passes over the egg towards the vegetative pole, separating the membrane from the egg. Just ('19) has given a careful description of the fertilization reaction in *Echinarachnius parma*. The reaction is similar to that in other echinoderm eggs. "The cortex reacts to penetration by pushing out a blister at the site of sperm entry." "From the point of sperm entry a definite gradient of membrane elevation is established, the last point of membrane elevation being at the pole opposite that of successful sperm entry."[1]

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[1] There seems to be a prevailing impression that Fol worked with compressed eggs. This is not, however, the case as Fol was at particular pains to state that the eggs were not in the least compressed ("sans les comprimer le moins du monde," '79, p. 176).

It therefore appears that in many eggs—echinoderms and lampreys—a change in the surface of the egg is initiated by contact of sperm and egg and that this change, which includes membrane separation and elevation, progresses from the point of contact in all directions to the opposite pole of the egg. It is probable that more careful observation would reveal the progressive character of the fertilization reaction in other eggs.[2] The wave-like progression of the reaction irresistibly suggests that electric phenomena are involved; recently Gray ('22) has made the same suggestion.

The roughening of the egg at fertilization has also been noted by a few observers. Schücking ('03) and Loeb ('13) record it for echinoderm eggs. The "peristaltic wave" which according to Okkelberg ('14) passes over the lamprey egg in normal fertilization or artificial activation is, I think, of the same character. Mr. Leigh Hoadley informs me that a roughening also occurs in the *Arbacia* egg on fertilization. The cause of the roughening is discussed later.

Flattening of the egg at the site of sperm entry has also not escaped observation and is regarded by some as a contraction. Hertwig ('78) probably has reference to this flattening when he states that in the starfish egg on fertilization "zieht sich der Dotter von der Eihaut zurück." Some of Fol's figures ('79) show this flattening and it is also recorded for *Toxopneustes* by Selenka ('78) and for *Petromyzon* by Calberla ('78). Schücking ('03) speaks of a contraction of the egg away from the vitelline membrane and probably has reference to the same phenomenon. The photographs of Ries ('09*a*) of fertilization in *Strongylocentrotus* plainly show the flattening at the site of initial membrane elevation and this is also mentioned by him in the text. Elder's drawing ('12, Fig. 6) of fertilization in *S. purpuratus* is similar. Gray ('16) speaks of the compression of the egg at fertilization by the contents of the perivitelline space.

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[1] Professor F. R. Lillie informs me that while at Pacific Grove in the winter of 1920 both he and his assistant, Mr. J. Nelson Gowanlock, observed the progressive character of the fertilization reaction in *Strongylocentrotus*.

[2] But not in teleost eggs, according to Reighard '93.

Watching the fertilization process one certainly gains the impression that the flattening of the egg is due to a pressure exerted on the egg by the contents of the perivitelline space. It appears that the filling of this space does not occur *pari passu* with the elevation of the membrane but is due to some other process. This suggests that the accumulation of materials in the perivitelline space is the direct cause of the elevation of the membrane and such an idea has been advanced by many investigators.[1] Further evidence on this matter is desirable.

4. The Identity of the Vitelline Membrane with the Fertilization Membrane.—This matter has been the subject of some dispute in the history of the fertilization problem. Harvey ('10, '14), McClendon ('11), Elder ('12), Loeb ('13), and recently Gray ('22) have expressed the view that the fertilization membrane is formed by the precipitation or coagulation of materials emanating from the egg on contact with the jelly or the sea-water. That the jelly is not concerned in the appearance of the fertilization membrane has been shown by Harvey ('14) and Lillie ('14, p. 553).

On the other hand the identity of the fertilization membrane with the preëxisting vitelline membrane of the unfertilized egg has been maintained by many investigators: Fol ('77, '79) for *Asterias, Sphærechinus*, and *Toxopneustes*, Hertwig ('78) for *Asterias*, Calberla ('78) for *Petromyzon*, Théel ('92) for *Echinocyamus*, Reighard ('93) for teleost eggs, Herbst ('93, '04) for *Sphærechinus, Echinus*, and *Stronglyocentrotus*, Delage ('01) for *Asterias*, Schücking ('03) for *Asterias* and *Strongylocentrotus*, Ries ('09*a*) for *Strongylocentrotus*, Allyn ('12) for *Chætopterus*, Glaser ('13) for *Arbacia* and *Asterias*, Heilbrunn ('13, '15, '20*b*) for *Arbacia* and *Cumingia*, Okkelberg ('14) for *Entosphenus*, and Chambers ('21*a*, '21*b*) for *Arbacia, Asterias*, and *Echinarachnius*. Most of these authors rest their view on direct observation of the elevation of the vitelline membrane as the fertilization membrane. Such evidence is not entirely satisfactory for the echinoderm egg as the vitelline membrane is not clearly visible on such eggs. Chambers' evidence appears to be conclusive. He has shown that if the vitelline membrane be removed from unfertilized eggs (*Arbacia*, *Asterias*, *Echinarachnius*), such eggs do not elevate membranes on fertilization. Further by various methods the vitelline membrane can be made more obvious on the unfertilized eggs at certain points and the continuity of these easily visible portions of the membrane with the fertilization membrane after insemination is easily observable.

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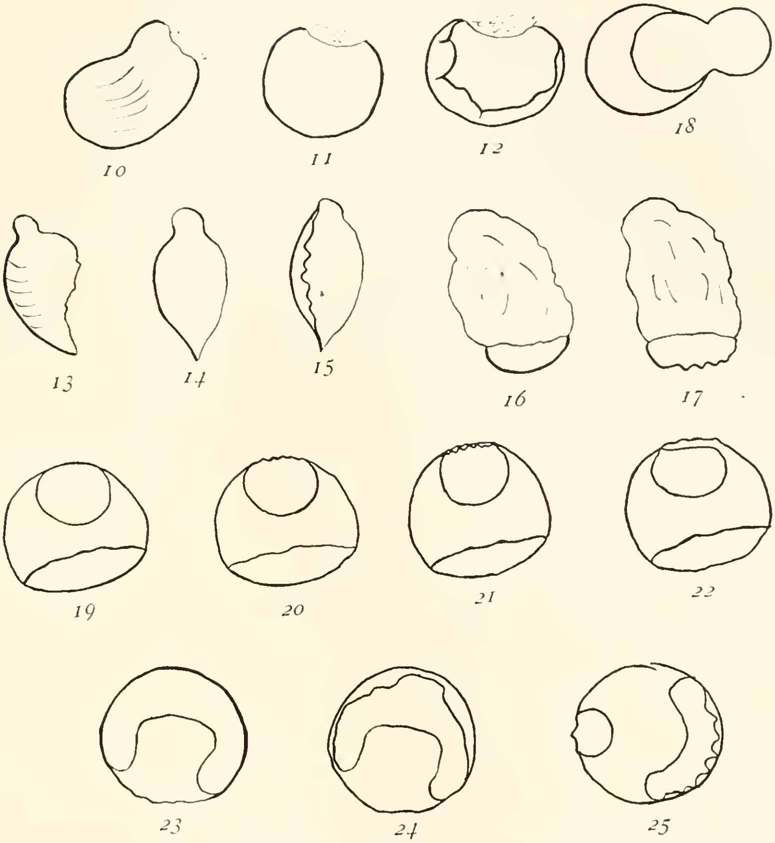
[1] *E.g.*, Herbst '93, '04, Schücking '03, Ries '08, '09*a*, Loeb '08. It seems clear that the contents of the perivitelline space consist chiefly of water which enters from the outside through the vitelline membrane. This was proved for the lamprey egg by Calberla ('78) by coloring the water and is certain for teleost eggs (Reighard '93). The perivitelline space also appears to contain some colloidal material probably of protein nature derived from the egg.

Further conclusive evidence of the identity of the vitelline and fertilization membrane is afforded by the study of the fertilization reaction in egg fragments. That portions of eggs may elevate fertilization membranes has been noted by Ziegler ('98), Moore ('12) and Glaser ('13). The fragments in these experiments were obtained by shaking the eggs and hence it is not known from which portions of the eggs they originate. It is certainly not justifiable to conclude from such experiments that any portion of the egg when isolated can reform a membrane capable of elevation since: (*a*) the cytoplasm being relatively fluid, much of it will be lost on rupture of the eggs and the cortical portion which adheres to the membrane will be most likely to persist; and (*b*) since it has been proved by Chambers ('21*a*, '21*b*) and Just ('23) that the interior of the egg is not fertilizable but only the cortex possesses this property, it follows that any fragments which fertilize must necessarily have retained a portion of the egg cortex and hence of the original vitelline membrane. It seems likely that most of the egg fragments obtained by shaking represent the whole cortex, the fluid interior having escaped.

I have studied the fertilization reaction in fragments of the eggs of all three species used. These fragments have been obtained by the method already described. An egg is held by surface tension and then punctured. The cytoplasm flows out and the egg begins to disintegrate. At various stages in this process the egg is rapidly pushed back into the interior of the drop. The outflow at once ceases when the pressure of surface tension is removed and portions of eggs of all sizes and shapes are obtainable. Such fragments of course always retain a portion of the original cortex and vitelline membrane and it is definitely known to the observer which is the original and which the new surface of the fragment. In *Patiria* the whole vitelline membrane clings to the fragment. The fragments show little tendency to assume the spherical form.

Upon the addition of sperm all such fragments give evidence of the fertilization reaction. The first change noted is an alteration of tension in the fragment. This is evidenced by the disappearance of the wrinkles from the vitelline membrane of fragments of urchin eggs and by a change of shape. The fragments tend to assume a more spherical form. This tendency towards sphericity is very marked in the larger fragments as in Figs. 10 and 11. From these observations the conclusion can be drawn that the change of tension is confined to the original cortex, since when the amount of cortex remaining is small there is much less change in the shape of the fragment than when it is large. The next change that is noted is the roughening of the surface. This occurs *only on that surface of the fragment which was the original surface of the egg.* In the smaller fragments the visible fertilization change stops at this point. The membrane follows the crenations of the surface as shown in Figs. 17 and 20; consequently the crenations cannot be due to the formation of small vesicles as supposed by Loeb ('13). In the larger fragments the roughening of the surface is followed by the elevation of the fertilization membrane. The membrane elevates *only on that surface of the fragment which was the original surface of the egg* and which therefore bears a portion of the original vitelline membrane. In the *Patiria* egg where the whole vitelline membrane remains attached to the fragments, the membrane elevated on the fragment is perfectly continuous with the empty portions of the vitelline membrane as shown in Figs. 19 to 25. The elevation of the membrane on egg fragments is illustrated in Figs. 10 to 25.

The fertilization reaction in eggs with extra-ovates is to the same effect. Glaser ('13) records that if the egg of *Arbacia* is ruptured so that part of the contents flows out, a fertilization membrane appears on one sphere but not on the other. According to my observations on the same egg, it is the cortical sphere which elevates a membrane. Chambers ('21*a*, '21*b*) also finds that extra-ovates in the starfish are not fertilizable and do not



Figs. 10 to 25. Fertilization of egg fragments. The original surface is indicated by a heavier line. Figs. 10 to 18, fragments of *S. franciscanus*. Fig. 10, large egg fragment showing wrinkles in the vitelline membrane; Fig. 11, same after insemination; the fragment is rounded and the wrinkles have disappeared; Fig. 12, same later, with membrane elevated on the original surface. Fig. 13, smaller fragment; Fig. 14, same after insemination, showing change of form and disappearance of wrinkles; Fig. 15, same, later, with fertilization membrane elevated on original surface only. Fig. 16, small fragment with entire membrane attached; Fig. 17, same after fertilization; only the characteristic crenations appear; the membrane does not elevate on such small fragments. Fig. 18, extra-ovate of *S. franciscanus* after fertilization; cortical sphere to left, endoplasmic sphere to right; only the former elevates a membrane.

Figs. 19 to 25. Fertilization of egg fragments of *Patiria miniata*. Fig. 19, small fragment with entire vitelline membrane; Fig. 20, same after fertilization, showing the characteristic crenations; Figs. 21 and 22, same, later, showing separation of the fertilization membrane; it is continuous with the vitelline membrane. Fig. 23, large fragment of *Patiria;* Fig. 24, same after fertilization. Fig. 25, large and small fragment left within the same vitelline membrane, shown after fertilization; the small fragment exhibits only the characteristic crenations; the large one has elevated a fertilization membrane.

elevate fertilization membranes but that these processes are confined to the cortical portion of the ruptured egg. He further states that if the extra-ovate remains in continuity with the cortical portion for some time it becomes fertilizable but still does not elevate a membrane. I have produced extra-ovates in *S. franciscanus* with the needle; upon fertilization the cortical portion elevates a membrane while the extra-ovate does not (Fig. 18).

From all of these observations it may be concluded: (*a*) that the vitelline membrane is identical with and is elevated as the fertilization membrane; (*b*) that the vitelline membrane is a definite morphological structure which cannot be reformed or replaced;[1] and (*c*) that the fertilization reaction, which includes other visible changes besides membrane elevation, is exhibited only by the cortex of the egg.[2]

Since the fertilization membrane is thus a preformed, preexistent structure, its separation from the egg at fertilization should be spoken of as membrane elevation and not as membrane formation. The advisability of this terminology was previously emphasized by Heilbrunn ('15).

When the vitelline membrane elevates as the fertilization membrane, however, it unquestionably undergoes certain physical and chemical changes. In the first place it would seem that the membrane must soften since in elevating it is distended considerably beyond its former circumference. A number of observers have attested that there is no decrease in size of the echinoderm egg at fertilization (Fol '79, Théel '92, Herbst '93, Schücking '03, Ries '08, Loeb '08, McClendon '10, Chevroton and Vlès '11, Gray '16, Chambers '21*b*). I was also unable to find any change in size of the eggs of *S. franciscanus* on fertilization.

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[1] Any portion of the egg cytoplasm does of course when exposed to sea-water form a protective surface layer which has no doubt physiological properties similar to those of the vitelline membrane but which is not elevatable by sperm. Several observers (Herbst, '03, Tennent and Hogue, '06, Harvey, '10, Heilbrunn, '20*b*) have recorded that a second membrane can be elevated after the elevation of the usual fertilization membrane by treatment with various agents (but not with sperm). This second membrane is in reality the hyaline layer, as also stated by Harvey ('14) and Heilbrunn ('20*b*).

[2] The importance of the cortex finds explanation on the basis of Lillie's fertilizin theory (cf. Lillie, '19, also Chambers, '21*b*). Just ('23) also shows that the fertilization reaction is confined to the cortex.

Consequently the vitelline membrane is distended at fertilization, and such distension indicates a softening or degelation of the membrane. According to R. S. Lillie ('15) the temperature coefficient of heat activation in the starfish egg indicates that a degelation process is involved. Just's ('22) recent experiments show that the membrane[1] becomes very much less resistant at the places where it is separating from the egg for such places burst when the egg is exposed to diluted sea-water during elevation. Thus the decreased resistance or softening of the vitelline membrane like other fertilization changes is initiated at the point of contact of sperm and egg and is transmitted from this point over the egg. Within a few minutes after elevation the membrane appears to toughen again. This hardening or toughening of the membrane after elevation has been spoken of by several observers. Thus Herbst ('93), Goldfarb ('13), C. R. Moore ('16), F. R. Lillie ('21) have recorded that the fertilization membrane of urchin eggs is much more easily removed by shaking within a few minutes after fertilization than later. Chambers ('21*b*) also finds that the fertilization membranes of *Asterias*, *Arbacia*, and *Echinarachnius* begin to toughen very soon after they are elevated. The extensive hardening of the fertilization membrane in nematode eggs is well known. That the membrane also undergoes chemical alteration on elevation may be inferred from an experiment of Harvey's ('10). He found that the vitelline membrane is soluble in concentrated sulphuric acid while the fertilization membrane is not.

5. *Viscosity Changes at Fertilization.*—It was pointed out in the first part of this paper that the cytoplasm of the unfertilized egg is a slightly viscous fluid. It can be determined by the same procedure as described there that at fertilization there is a sudden and marked increase in viscosity. In the eggs of *Strongylocentrotus* (both species) this increase in viscosity occurs just before the membrane begins to elevate; this is probably also true of the *Patiria* egg although it was not determined with certainty. It is present in this egg at least as soon as the membrane lifts at the site of sperm entry. The viscosity increase is

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[1] Just speaks only of the softening of the cortex but probably includes the membrane in this term. It seems evident that the membrane must also be involved in the softening otherwise it would not yield at the places in question.

well marked and occurs very suddenly, at the moment before membrane elevation is initiated. At this moment, the cytoplasm flows less readily than before and cuts and gashes made with the needle close up more slowly. The viscosity increases during the elevation of the membrane but appears to reach a maximum in a very short time.

We may therefore speak of an increased viscosity or gelation of the egg cytoplasm as one of the changes included in the fertilization reaction. I believe this gelation to be responsible for the roughening of the egg surface at fertilization. As Rhumbler ('05) has recognized, a rough and crenated surface is causally related to a solidified condition of protoplasm. As the roughening of the egg begins at the point of contact of egg and sperm and spreads from there over the egg, it follows that the gelation process must likewise originate at the site of sperm entry and progress from this point in all directions. It seems probable that the gelation process is confined to the cortex of the egg since in egg fragments only the original surface roughens at fertilization.

The interpretation of the rounding up of egg fragments remains to be considered.[1] I at first thought this to be another indication of increased viscosity, of the increased tension accompanying the change from a more fluid to a more viscous state. But obviously gelation cannot cause both a rounding up and a roughening. Although the surface tension of gels is higher than that of sols, still surface tension is not great enough to induce sphericity in fragments of gels. It therefore seems necessary to conclude that the rounding of egg fragments indicates a decreased viscosity or increased fluidity. As my observations show that this process precedes the roughening by a quite perceptible time interval, it seems that the cortex of the egg at fertilization first becomes more fluid and then undergoes gelation. Just ('22) emphasizes a liquefaction of the cortex as part of the fertilization reaction.

The gelation of the egg at fertilization serves at least two purposes: (*a*) the vitelline membrane is split from the egg cytoplasm and acquires a definite internal boundary which it had hitherto lacked;[1] and (*b*) a new surface is formed on the egg cytoplasm, the so-called hyaline layer, which prevents the egg contents from expanding with the membrane and which replaces physiologically the vitelline membrane. The separation of the vitelline membrane from the egg cytoplasm at fertilization thus appears to me to be a process quite independent of the subsequent elevation of the membrane. The hyaline layer which replaces the vitelline membrane requires some time for its complete development and is according to Chambers ('21*b*) "firm and gelatinous."

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[1] Harvey ('10) has also emphasized the rounding up of eggs at fertilization and attributes it to an increase in tension. However, the same tension will induce sphericity if the cytoplasm becomes more fluid. My observations on egg fragments indicate that this change in tension is in the cortex, not in the membrane.

An increased viscosity accompanying the fertilization reaction has not been hitherto recorded. Several observers have, however, noted such an increase following after fertilization, recently Heilbrunn ('15, '20*a*, '21), Chambers ('17*b*, '19), and Seifriz ('20). According to these investigators the increased viscosity is associated with some phase of the mitotic figure. According to Chambers ('17*b*) the gelation after fertilization is at first limited to the small sperm-aster and later spreads throughout the egg. While fully accepting the conclusion of these authors that asters and spindles are gelation figures I do not think that the initial gelation which constitutes part of the fertilization reaction is due to the sperm aster. The latter is at first localized around the sperm head while the gelation which I am considering appears to be general throughout the whole cortical region of the egg.

Since the viscosity changes at fertilization precede the elevation of the membrane it may be emphasized that the latter is a secondary rather than a primary phenomenon in the fertilization reaction. It seems certain that changes have taken place in the egg before the membrane elevates. This has recently also been emphasized by Just ('19): "In the *Echinarachnius* egg, normal development has already been initiated by the sperm when the membrane begins to form." It appears that the elevation of the membrane is not due directly to sperm penetration but is the result of changes in the egg.

6. Artificial Membrane Elevation and Cytolysis.—It is well known that a number of chemical substances as well as other agents will induce a fertilization membrane in echinoderm eggs. This matter has been discussed from the point of view of membrane formation by Traube ('09), Loeb ('13), Gray ('22) and others and from the point of view of membrane elevation by Heilbrunn ('13, '15). I have made a few observations on artificial membrane elevation, using chiefly ether and diluted sea-water or distilled water but also butyric acid. In employing ether and similar substances for membrane elevation, the eggs must be rapidly returned to normal sea-water, as noted by Loeb ('13) if any are to be saved from cytolysis.

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[1] Fol ('79) in particular emphasizes that the vitelline membrane (couche enveloppante) lacks a definite internal boundary and that it acquires one at fertilization.

After the application of membrane-elevating substances such as ether and distilled water, three classes of eggs are noted: those with blister-like elevations, those with completely elevated membranes, and those which are cytolyzed. In a considerable number of eggs after treatment with ether and distilled water, the membrane is elevated only as local blisters. In one or two cases such blisters were observed to spread over the egg until the entire membrane was elevated but generally they persist unchanged as long as observed (unless cytolysis intervenes). They appear to be due to chance inequalities of contact with the membrane-elevating solution when the latter is first applied and indicate that the local action of such solutions is incapable of inducing complete membrane elevation. Such partially elevated membranes can be completed by sperm. The portions elevated by sperm appear to be continuous with those elevated by the agent.

After treatment with ether and distilled water there is obtained a small percentage of eggs in which the membrane is completely elevated and which cannot be distinguished visibly from eggs fertilized by sperm. Investigation with the needle shows, however, that as concerns viscosity conditions these eggs are entirely different from normally fertilized eggs. Whereas in the latter, as already noted, the cytoplasm has undergone gelation, in these eggs with artificially elevated membranes there is no trace of such increased viscosity. These latter eggs are as fluid as or more fluid than normal unfertilized eggs. This agrees with Heilbrunn's ('20*c*) statement that ether, chloroform, and similar substances liquefy the *Arbacia* egg. It is thus evident that the response to membrane-elevating substances, so far as tested, is not equivalent to the response to sperm.

The usual butyric acid treatment yields, after return to normal sea-water, a percentage of eggs with membranes of normal appearance equal to that produced by sperm. But in such eggs, also, the normal gelation appears to be lacking.

The majority of the eggs treated with membrane-elevating substances such as ether and distilled water undergo a change designated by Loeb as cytolysis.[1] This condition is sufficiently described in Loeb's book ('13). The eggs are much expanded and transparent. It appears from Loeb's description ('13, p. 188) that he regards this cytolysis to consist in an absorption of fluid followed by a liquefaction of some of the egg contents. It can readily be shown by the needle that this conception of cytolysis is erroneous. Cytolysis is not a liquefaction of the egg contents; it is a complete and irreversible coagulation. This fact was discovered by Heilbrunn ('15) and the increased viscosity of cytolyzed eggs was also noted by Goldfarb ('18). When cytolyzed eggs are punctured with a needle, a small amount of watery fluid generally escapes (this is probably water which passes in from the outside when the membrane expands) but the egg material itself will no longer flow. It is completely solidified and can be cut into pieces with a needle.

The coagulation [2] caused by membrane-elevating solutions is entirely different from the normal gelation attendant on fertilization. The cytolytic change is an irreversible lethal change in which the egg colloids are precipitated out in a coagulated mass. The viscosity of the cytolyzed egg is very much greater than that of the normally fertilized egg at any time from fertilization to the first cleavage. The normal gelation on the other hand is a reversible physiological process in which there is no such precipitation of colloids.

The cause of cytolysis appears to be as follows. Since after treatment with membrane-elevating solutions without cytolysis, the membrane elevates without an accompanying cytoplasmic gelation, it is evident that the egg is left without a resistant surface. Consequently the surface of the egg after lifting of the vitelline membrane is weak. The membrane-elevating substances tend to induce the expansion of the egg in the same way as they induce the expansion of the membrane (cf. further Heilbrunn, '15) and as the egg is not protected by a resistant surface it naturally ruptures by expansion. Such rupture causes coagulation since it has been shown by the microdissectionists (Chambers, '17*a*, Seifriz, '20) that injury leads to coagulation. The agents in question do not cause coagulation directly but act by elevating the protective vitelline membrane from the egg, leaving the cytoplasm without a sufficiently resistant surface.

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[1] My remarks refer only to the "white" cytolysis of Loeb.

[2] The term coagulation can of course be used to designate any marked increase in viscosity. It seems preferable to me, however, to confine the term to an irreversible separation out of colloids and to use the term gelation to designate reversible physiological increases in viscosity.

From these considerations it is highly questionable whether the cytolytic action of parthenogenetic agents has any relation whatever to their parthenogenetic power or whether any conclusions can be drawn concerning the mechanism of normal activation from the cytolytic properties of such agents. A similar conclusion as to the lack of relation between cytolysis and activation has been reached by Just ('20) from other lines of evidence. It seems sufficiently evident that cytolysis is simply a death change and has no bearing on activation.

It occurred to me to determine whether artificial agents can elevate membranes on eggs in which the sperm are not able to do so. It is well known that after standing in sea-water for twenty-four hours or more urchin eggs no longer elevate membranes on fertilization although they are still capable of development. The vitelline membrane probably loses its elasticity and capacity for distension after a prolonged stay in sea-water. I found a considerable degree of parallellism between the action of sperm and of artificial agents on such eggs. It is much more difficult and in some cases impossible to induce membrane elevation by agents in eggs which do not elevate membranes on insemination. In most cases, however, the artificial agents are more or less effective in partially or completely elevating the membrane. The action of these agents is thus more powerful than that of the sperm and may possibly be of a different nature.

In some cases membrane elevation could not be induced by artificial agents in these stale eggs. In such cases the egg bursts through the membrane, forming either a number of vesicles or erupts after the manner of an extra-ovate. This further indicates that the agents used have an expansive effect upon the egg contents as well as on the membrane. When extra-ovates are formed from stale eggs by these agents the vitelline membrane separates from the egg contents. As this is not the case when extra-ovates are induced in fresh eggs, one may again conclude that the vitelline membrane loses its elasticity and distensibility on standing. The membrane appears much stiffer and firmer than in fresh eggs; in all probability it is coagulated.

7. *Conclusion.*—In conclusion I may be permitted to reiterate an old view that the activation of the egg by the sperm is of the nature of a stimulation or excitation. Among the characteristics of a stimulation are: (*a*) many different agents are capable of exciting the same effect in the protoplasm which is stimulated so that there is no specific relation between the properties of the agents and the change invoked in the protoplasm; (*b*) the changes induced in the protoplasm stimulated are altogether in excess of the energy content of the stimulus; (*c*) the changes invoked in the stimulated protoplasm depend upon the constitution of the protoplasm and not upon the nature of the stimulus; *d*) the excitation is transmitted from the point of application of the stimulus. It is evident that the activation of the egg exhibits these characteristics. Many different agents are able to induce activation. There appears to be no specific relation between the changes induced in the egg and the physical and chemical properties of the stimulating agents. To suppose that the sperm brings into the egg some substance which evokes the activation changes is I think just as far from the truth as to suppose that the various agents which induce a nerve impulse do so by injecting some substance into the nerve. I think one must agree fully with Lillie ('19, Chap. VII) that the egg is "an independently activable system" and "possesses all of the substances necessary for activation." Finally it has been shown in at least a number of cases that the fertilization reaction is initiated at the point of sperm entry and is transmitted from this place over the egg. The

transmitted changes recorded in this paper are: the roughening (gelation) of the egg and the elevation of the fertilization membrane. To these may be added those recorded by Just ('19, '22)—the loss by the egg of fertilizibility, the passage of materials from the cortex into the perivitelline space, and the softening of the vitelline membrane.

The problem of the activation of the egg becomes thus a problem of the nature of stimulation in general and cannot be solved until the more general problem has attained solution.

8. Summary.—(*a*) The eggs used were those of *Strongylocentrotus franciscanus* and *purpuratus* and *Patiria miniata.*

(*b*) Physically these eggs consist of a slightly viscous cytoplasm inclosed in a vitelline membrane of solid consistency.

(*c*) In all three species the fertilization reaction begins at the point of attachment of the successful sperm and is transmitted from this place in all directions over the egg.

(*d*) The visible manifestations of the fertilization reaction are a roughening of the surface and the elevation of the vitelline membrane. Both begin at the site of sperm entry and spread from there over the egg.

(*e*) The vitelline membrane is identical with and is elevated as the fertilization membrane.

(*f*) In fragments of eggs only that surface of the fragment which was part of the original surface of the egg shows the fertilization reaction-roughening and membrane elevation.

(*g*) The vitelline membrane cannot be replaced or reformed.

(*h*) At the moment of fertilization just preceding the elevation of the vitelline membrane an increased viscosity or gelation occurs in the egg. This splits the vitelline membrane from the egg cytoplasm and provides a new resistant surface for the latter. This gelation is the cause of the roughening of the egg at fertilization.

(*i*) A change in the egg thus precedes membrane elevation and makes it probable that the latter process is not the primary event in the fertilization reaction.

(*j*) After membrane elevation by such artificial agents as were tested there is no increased viscosity in the egg but the cytoplasm is of the same or of less viscosity than the unfertilized egg, until cytolysis occurs.

(*k*) Cytolysis consists of an irreversible coagulation of the egg cytoplasm and appears to be due to the fact that the egg after the action of cytolytic agents is left without a resistant surface. It consequently ruptures and this injury leads to coagulation. Probably cytolysis bears no relation to activation.

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BIOLOGICAL BULLETIN

THE ORIGIN OF THE MYCETOCYTES IN PSEUDOCOCCUS.

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Introduction.

Symbiotic fungi or mycetozoa which are to be found in many species of insects, have lately been made the subject of some interesting work chiefly in Germany and Italy. This peculiar association between fungus and insect body seems especially well developed in the Homoptera and there a great variety of special features is to be found in its development. In some cases, among which are the mealy bugs of the genus Pseudococcus, the symbionts are lodged in or associated with cells that originate in the insect body—the mycetocytes. These cells have a peculiar interest in that they are evidently very much specialized and are restricted to a very definite locality in the body of the insect. In all of the Homoptera, the symbionts are transmitted from one generation to the next through the eggs; each of which receives a certain number of the fungi from the mycetome or symbiont mass of the mother. In *Pseudococcus*, such a transfer to the ova involves a dissociation of the symbionts from the mycetocytes, for the latter do not pass into the eggs. When the infection of the egg is complete, the fungi are therefore found "naked" near the anterior pole and always in the form of a number of spherical clumps or packets, each of which contains a large number of the symbionts. During the development of the embryo, these clumps once more become associated with mycetocytes which arise in some way in the embryo.

The exact nature of the mycetocytes has received a variety of interpretations. Breest ('14), working on *Aspidiotus*, a coccid not distantly related to *Pseudococcus*, suggested that they arise from the yolk cells—that is, cells that remain behind in the yolk at the time of blastoderm formation.

Strindberg ('19), who worked on *Lecanium*, reported that in that coccid also, the mycetocytes took their origin in the yolk cells.

Pierantoni, who was the first worker to make a detailed investigation of *Pseudococcus* gives a different account of the mycetocytes ('10, '11, and '13). According to him, some of the cleavage cells in traveling to the periphery to establish the blastoderm, encounter the symbionts. This association, more or less accidentally initiated, becomes permanent, and the cleavage cells assume the characters that stamp them as mycetocytes. Exactly the same process has been described by Pierantoni also in *Icerya*.

Buchner ('21) in reviewing previous investigations, seems inclined to agree with Pierantoni.

Finally, Shinji ('19), also working on *Pseudococcus*, describes a migration of cells to the symbionts, shortly before the germ band commences its growth. These migrating cells he interprets as potential germ cells. Some of them become permanently associated with the symbionts and constitute the mycetocytes, but others migrate once more to form the definitive gonads.

In criticizing these varying results, I am not in a position to pass judgment on the conclusions of Breest and Strindberg. Certainly my own results in regard to the point in question, *i.e.*, the origin of mycetocytes, have led me to an entirely different interpretation.[1] But this difference may very well be due to an actual difference in the development of mycetocytes in the three genera under consideration.

The work of the other investigators mentioned has already been considered in a recent paper ('23). Although a considerable part of that paper was devoted to showing that Shinji's conclusions are untenable, I also took up briefly the statements of Pierantoni. My own position on this question of the nature of the mycetocytes can best be presented by giving a short resumé of those of my findings that are involved in the present discussion.

In both *Pseudococcus citri* and *P. maritimus*, the somatic number of chromosomes is ten, all being alike in size and shape (Figs. 1–3). Five tetrads are formed in the maturing egg, and the egg nucleus undergoes a reduction and an equation division. The order in which these two divisions occur cannot be ascertained. The first division results in two daughter groups each consisting of five dyads. One of these groups of course represents the first polar body. It is not extruded but remains in an inactive state at the periphery. The other group of dyads undergoes the second maturation division, the results of which are two groups, each containing five unit chromosomes or monads. One of these last two groups remains at the periphery, constituting the second polar body. The other sinks into the egg and there combines with the male pronucleus.

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[1] Regarding other points in the early development, I am in essential agreement with Strindberg.

The five dyads of the first polar body now break up into their unit elements, ten in number, and then enter the resting condition. The five monads of the second polar body also become diffuse. The two polar bodies then approach until in touch with each other. Fusion may then actually occur and I have described such a case in my previous paper ('23). In other cases however fusion is delayed until the chromosomes of each have been almost completely reformed and the nuclear walls begin to break down. The chromosomes then intermingle to form a single group, and this of course contains 15 chromsomes. With this act of combination the polar nucleus becomes established (Fig. 4).

The polar nucleus undergoes two or three divisions which appear normal in every respect (Fig. 4 to 7). Following the last of these divisions, irregularities occur, and in the course of these the chromosomes are greatly increased in number and the size of the cells is enlarged. These phenomena occur in every egg and the resultant cells I have called giant cells. The giant cells once established, undergo some apparently normal divisions and then become separated from the periphery and migrate a considerable distance through the egg to the symbionts. With these they enter into association and thus form the mycetocytes, which in this manner are formed anew in every embryo.

Although this account portrays the general course of events, it leaves unexplained the exact nature of the irregularities which convert the derivatives of the polar nucleus to giant cells. It is this point with which the present paper is concerned.

The work was done on two species, *Pseudococcus citri* and *P. maritimus.*

Chromosome Counts.

The short statement regarding the origin of giant cells was given as follows in my previous paper ('23): "Later divisions of the polar nucleus derivatives are subject to irregularities. Apparently nuclear division is then very often or even generally not accompained by cytoplasmic division, so that the two resulting nuclei may lie side by side in a single protoplasmic area. At the ensuing division, there may be an intermingling of the chromosomes evolved, or else a multiplicity of spindles. Possibly also, cleavage cells nearing the edge may at times fuse with the derivatives."

This statement covers the problem only in a very general way and is hardly definite enough to be regarded as a solution of the complexities that are to be observed in the behavior of these peculiar cells. The one point established is that the polar nucleus derivatives are involved in some way in giving rise to the giant cells.

Without making any reference to this early period in the embryology of *Pseudococcus*, Buchner ('21) in his work on symbiosis comments on the fact that the mycetocytes in the adult contain a multiple number of chromosomes. This condition I had also observed, but it was not until recently, when the embryology was worked out, that the relationship between the giant cells of the embryo and mycetocytes in the adult became clear to me. Buchner apparently did not study the younger stages of *Pseudococcus* and therefore did not observe the giant cells at all.

The giant cells when first making their appearance in the egg are marked by a peculiarity that was observed very early in my investigations. This is, that although the number of chromsomes they contain is clearly variable and always greater than the true somatic number, it nevertheless varies only within very definite limits. This led to a more careful examination of the chromosome numbers in these cells.

A large number of chromosome plates was investigated. Unfortunately, the large size of the cells and plates often results in their being cut in the process of sectioning. Even if the chromosome plate studied is entire and flat, the chance of an overlapping of several chromosmes is very great, considering the large number involved. The latter defect may often make a count unreliable, but the cutting of a plate must throw it out of consideration at once. Discarding therefore all counts in which any doubt as to the number can possibly be entertained, the following data were obtained:

There are 6 plates containing 25 chromosomes; 3 plates containing 30 chromosomes; and 5 plates containing 35 chromosomes. These numbers become more impressive in view of the fact that during this period not a single perfect plate carrying any other but these three numbers was encountered (Figs. 12–17).

Among the plates discarded because overlapping of chromosomes made their counting uncertain, there were several in which doubt existed only with respect to a single chromsome, *i.e.*, whether to count it as one or two. Accordingly as these two possibilities were taken, these doubtful plates were found to fall under one of the three types given, or differ from it by one chromosome. In this uncertain group of plates there are 5 plates containing 25, or 1 more or less chromosomes; 3 plates containing 30, or 1 more or less chromsomes; 1 plate containing 35 or 36 chromosomes. Again, no plate in which doubt as to one chromsome exists was found to approximate any number but 25, 30 and 35.

How are these numbers to be explained?

In every perfectly clear plate, it was evident that the chromosomes resemble each other very closely as to size and shape. This fact coupled with the observation that the multiple numbers found are all multiples of five makes it improbable that an irregular process of fractionation of the chromosomes is to be held accountable. Again, although a definite regularity obtains in these chromsome numbers, it has also been observed that one egg may carry cells of more than one type. Thus a plate with 25 may be found close to another plate with 35 chromosomes. This too would be difficult to explain on the basis of a fractionation, especially as the character of the numbers would make inevitable the hypothesis that only certain definite chromosomes break up into smaller units.

If effective polyspermy were common, the possibility that the supernumary sperms might combine with each other or any of the embryonic cells should receive some consideration. It would then remain to be explained why such numbers as 20 or 40 should not be encountered, since the 5 chromosomes of each sperm should make almost any combination possible. But aside from this consideration, I may point out as I have previously done, that supernumary sperms even if present rarely evolve chromosomes and certainly not as part of a regulated process, whereas giant cells with their multiple numbers of chromosomes are to be found in every developing egg at the right stage.

Finally, as the last of these possible but unlikely hypotheses remains an irregular behavior of the polar bodies. It is perfectly possible that the first and second polar bodies may not combine and that one or both may continue to divide independently. In combination with embryonic cells, the second polar body might thus bring about such numbers as 25 and 35. But against this I need repeat only my former observations that in all eggs at the crucial stage under observation, the polar nucleus is always formed and its 15 chromosomes go through several normal mitoses. None at all show an independent development of the two polar bodies. This of course still leaves the possibility that the first polar body may at times divide before fusion with the second. It may even be admitted that independent divisions or development of the polar bodies may sometimes occur. But such cases, and I have not seen any, are not normal.

These considerations leave only two types of cells as factors in the origin of the giant cells. They are the polar nucleus derivatives carrying 15 chromosomes and the true cleavage cells with 10 chromosomes.

Taking up the three types of giant cells in order, it is plain that the 25 chromosome type can arise only by a combination of a polar nucleus derivative (15 chromosomes) with one cleavage cell (10 chromosomes).

The 30 chromosome type must arise from a fusion, or recombination after mitotic division, of 2 polar nucleus derivatives.

Numerically, the same number could be attained by the combination of 3 cleavage cells. The latter possibility is more than doubtful if it is considered that giant cells are formed only in a certain and limited area at the periphery, whereas if a fusion of cleavage cells alone is a possibility such cells with 30 chromosomes should then be formed at any part of the periphery where cleavage cells are forming the blastoderm. Weighty although negative evidence against this hypothesis is to be adduced from the seeming nonexistence of giant cells with 20 chromosomes. The failure to find such cells is all the more significant if it is considered that the chances of two cleavage cells coming together for fusion are greater than the chances of three combining in that way.

The 35 chromosome type can originate only from a combination of a polar nucleus derivative with two cleavage cells. Other chances of combination to bring about this type have been ruled out by the preceding considerations.

Why a 40 chromosome cell should not be found at this stage it is not possible to say. Possibly there are such cells but their less frequent occurrence has prevented their discovery. More probably the three types already mentioned represent all the combinations possible.

The Giant Cell Chromosomes at Later Stages.

As already mentioned, the giant cells originate always at the time that the cleavage cells are migrating to the periphery of the egg to establish the blastoderm. With the completion of the latter and the first stages of germ band formation, another period in the history of the giant cells is initiated. In the course of this, giant cells with still greater numbers of chromosomes than those already described, are encountered. Beside them, the three older numerical types may continue to exist. The increase in the number of chromosomes, together with the fact that divisions in the giant cells tend to decrease their size, make certain counts in these later cells a much more difficult matter. Apparently most of these greater chromosome numbers hover in the neighborhood of 60 or 70. Only one certain count could be made, that being of a plate containing 60 chromosomes (Fig. 18).

Cytological evidence to be considered later, makes it probable that the period of fusion has now been passed. It is indeed possible that there is an occasional combination of a giant cell with one of the yolk cells, the latter representing nothing but cleavage cells that failed to migrate to the periphery to establish the blastoderm. Fusion with what were formerly cleavage cells but must now be termed blastoderm cells, can of course take place only at the edge of the giant cell area, where the two types of cells are in contact. But aside from the fact that the giant cells in that location are not larger as a rule than those more centrally placed, it must be considered that the blastoderm cells in their expansion seem to exert actual pressure on the giant cells. The latter are heaped up and finally actually leave the periphery altogether. If such a pressure really exists, it would be constantly cancelled by fusion of adjoining giant and blastoderm cells.

Nor can there be a continued tendency of giant cells to fuse with each other. Such occurrences are at least not general, for the number of giant cells is at this time slowly but steadily increasing, while their individual size is decreasing. Nevertheless the size is evidently variable, so that an occasional division of the chromosomes unaccompanied by cytoplasmic division remains as the most plausible explanation.

Once the giant cells have migrated to the symbionts and entered into association with them, divisions become rarer. At the same time it must be observed that in the adult *Pseudococcus*, the former giant cells, then called mycetocytes, contain relatively enormous numbers of chromosomes. The association with the symbionts must therefore have a disturbing effect on the few divisions that still occur, and most probably it is the failure of cytoplasmic division following a normal division of the chromosomes that thus causes a multiplication of the chromosomes. This idea has already been expressed by Buchner ('21).

Cytological Evidence.

A complete cytological consideration of the problem should begin with a study of the maturation phenomena in the egg. Since these primary steps have already been considered at some length in my previous paper ('23), it is sufficient to begin the present account with the polar nucleus, *i.e.*, the combined first and second polar bodies. The first difficulty arises in determining the number of divisions to which the polar nucleus is subjected. It seems certain that at least two divisions take place regularly and that they are always normal. In some cases, the 4 nuclei or polar nucleus derivatives resulting from these two divisions certainly undergo a third division. But whether this last division occurs in every egg is not so certain. If so, there will then be 8 polar nucleus derivatives at the periphery of the egg (Fig. 4–7a).

Almost the same difficulties are encountered in determining the number of divisions that the fertilization or zygote nucleus undergoes, before the resultant cleavage cells take up their migration to the periphery to establish the blastoderm. Typically there appear to be about 32 cells in the interior of the egg when the migration begins.

It will be apparent that there is a distinct variation in the rate of division of polar nucleus derivatives and cleavage cells respectively. While the polar nucleus is undergoing at most 3 divisions, the fertilization nucleus undergoes approximately 5. The result of this is that while all the nuclei of each type taken by itself are at about the same stage of division, they may not be at all synchronous with the division stages of the other type. It is this condition that lies at the bottom of the difficulty in determining the number of divisions that the nuclei in question undergo before the process of fusion is begun. Thus in one egg, there are 8 polar nucleus derivatives, all in slightly varying stages of telophase, and still connected in pairs by spindle fibers (Fig. 7). The cleavage cells of this egg have begun the peripheral migration, but none have yet reached the edge. It can be assumed that here 8 polar nucleus derivatives will be involved in the processes of fusion to follow. In contrast with this is another egg in which there are only 4 polar nucleus derivatives. The chromosomes are in the final stage of condensation but the nuclear walls have not yet been broken down. The cleavage cells, present in about the same number as in the previously mentioned egg (32), are again in the stage of migration, and one has actually come in touch with one of the polar nucleus derivatives at the periphery. This cleavage cell like its sister cells is in the resting phase. The question therefore arises whether the polar nucleus derivative will complete its impending division regardless of the proximity of the cleavage cell, or whether the presence of the latter will make that division abortive. In the first case, 8 nuclei will commence the fusion process as before; in the last named eventuality only 4 will be at hand (Fig. 7*a*).

On the whole it may be assumed that the polar nucleus as well as the fertilization nucleus undergoes a definite number of divisions. As has been noted previously, the chromosomes of any single giant cell are from their first appearance alike in size and shape. The chromosomes of the polar nucleus derivatives however decrease in size with each succeeding division (Fig. 4 to 6) and the same is true of the cleavage cells. If fusion or combination of these two types of cells could occur after a varying number of divisions, it would be expected that the chromosomes of the combination nucleus would often be of two sizes. But this, as has been said, is not the case. It is of course possible that there is some regulative mechanism capable of equalizing differing sizes of chromsomes, but for this assumption there is little or no basis.

Regarding the phenomena of fusion which now occur, the conclusions based on the numerical data receive the full support of the purely cytological evidence. In giving this last named proof I am fully aware of the ease with which in a case like the present, a number of isolated figures can be seriated to fit a preconceived hypothesis. Standing alone, the cytological proof would therefore be advanced with considerable caution. Nevertheless one or two of the figures found are of considerable value in themselves.

Every step in the migration of the cleavage cells to the periphery, their approach to the polar nucleus derivatives, the flowing together of the protoplasmic areas which surround each nucleus and the final apposition of the nuclei within the single protoplasmic area resulting, can be traced through closely seriated stages. Similarly, what appears to be a pair of polar nucleus derivatives may at times be seen in close proximity. However unless the chromosomes of the apposing nuclei are close to full condensation, no definite conclusion can be reached as to the nature of the nuclei involved in either case. All of the figures show that the process takes place in either two or three cells, and a greater number has not been observed (Figs. 8 to 10).

It might be supposed that even during the resting phases the sizes of the fusing nuclei would suffice to identify them. And indeed it seems well established that when the two types of nuclei are at precisely the same stage, that of the polar nucleus derivative with its 15 chromosomes is slightly larger than a cleavage nucleus with 10. But it is practically impossible to exactly identify the phase of the nucleus during its preparatory phases. At the same time it has already been noticed that variations in the size of any one type of nucleus are extreme. The changes in size seem directly related to the condition of the contained chromatinic material and are such that the nuclear volume is smallest just after the formation of the nuclear wall at telophase, and largest immediately before the dissolution of the nuclear wall prior to the following division. Thus the increase in size of the female pronucleus between the telophase of the last maturation division and the time when the chromosomes are again almost fully condensed before the first segmentation division, are very considerable (Figs. 8*a* and 11, '23). Changes of size almost as great can be observed in the polar bodies prior to the formation of the polar nucleus and the polar nucleus derivatives. It is therefore manifestly impossible to arrive at any conclusion regarding the nature of fusing nuclei by simply comparing their size when it is considered that a further complication arises from the fact that apposing nuclei may be at entirely different phases (Fig. 7*a* and 10).

In spite of the very different phases that two or even three apposed nuclei may be in, it is apparent that a normal plate of chromosomes, which represents the summation of the numbers contained in each of the fusing nuclei, is finally attained. This may happen only when all of the nuclei are in a very definite generation of cells as has been pointed out in regard to the question of the number of divisions undergone by the polar nucleus. It is also a consequence of these observed facts regarding the varying phases of apposed nuclei, that the chromosomes of one or two of the nuclei will reach their full condensation prior to those of the other nuclei involved. Those first evolved must therefore be subjected to a suspension of further activity until those lagging behind have caught up. All of my figures make it plain that condensation of chromosomes progresses regardless of the phase of an apposed nucleus, and that therefore the period of suspension of activities occurs when the chromosomes have been fully evolved.

Whether complete fusion of such nuclei is ever brought about before the condensation of chromosomes cannot be answered with certainty. A cytological demonstration would be next to impossible if the act is a very short one—say like the fusion of two soap bubbles to make a single larger one. That I have no stages showing such an act is therefore not complete proof that it does not occur. Nevertheless the normal course consists of a condensation of the chromosomes entirely independent of any other nucleus, and the fusion occurs only when the nuclear walls break down and permit an intermingling of all the chromosomes.

It is owing to the conditions brought out in the preceding paragraphs that a very good cytological demonstration of the act of fusing can be given. In Fig. 10 are shown three nuclei in apposition, and in the light of the numerical data they may safely be assumed to represent one polar nucleus derivative and two cleavage nuclei. Without the numerical data however, no such assumption would be justified. Fig. 11 on the other hand furnishes strikingly independent proof. Here there are 20 chromosomes almost fully condensed, and these show a slight trace of being arranged in two groups of 10 each. But in addition there are 15 chromosomes still in a more threadlike stage, and evidently at an earlier phase of condensation. The figure evidently represents a case in which the nuclei when coming into apposition were at different phases. The conclusion seems inescapable that here is represented the fusion of a polar nucleus derivative with two cleavage cells.

Spindles formed in the first division of these combination or fusion nuclei are apparently perfectly normal. Multipolar spindles are indeed encountered but little if any more frequently at this time than they are in the normal tissue of many animals. I am entirely at a loss to explain how the mitotic mechanism of two or even three combining nuclei is adjusted to the process of fusion. Certainly all the involved nuclei are capable of dividing perfectly independently. Bowen ('22) has recently pointed out a similar case in *Loxa florida* where a fusion of cells is likewise unaccompanied by any irregularity in the mitotic spindle formation.

Conditions in Older Embryos and in Adults.

As explained previously, the fusion process is limited chiefly to the period in which the blastoderm is laid down. When the latter is fully established, figures showing apposed nuclei in a single cytoplasmic area become very rare. Most of such figures arise from what is probably an accidental migration of yolk nuclei to the periphery, for a few have been found in the blastoderm as well as in the giant cell area. It is even doubtful whether in these isolated cases a fusion of nuclei is finally consummated, for no multiple cells have been found in the blastoderm cell region. The increase in the numbers of chromosomes in the giant cells, which is certainly still occurring at this time as well as later, is therefore due principally to a division of chromosomes unaccompanied by a cytoplasmic division of the cell concerned (Fig. 18).

When after leaving the periphery the giant cells have become associated with the symbionts, mitotic figures are not so often found in them. Nevertheless they do occur, and successfully as far as the chromosomes are concerned. Cytoplasmic division which before the migration, was undoubtedly completed successfully in some of the mitoses, is now completed less often. Only in this way can the relatively enormous numbers of chromosomes in the mycetocytes of the adult be explained. Further fusion is in these later stages practically eliminated, since most of the mycetocytes are almost completely hedged in by the symbiont spheres which they harbor.

Buchner ('21) estimates the chromosome number in some of the mycetocytes as over 200. In this estimate I can only concur with him. There is in addition to this a decided increase in the individual size of the chromosomes, although this seems to be a variable feature in different mycetocytes (Fig. 19 and 20).

My material is not favorable for a detailed study of the spindle formation in these later mycetocytes. The centrosomes seem extremely small under even the most favorable circumstances, and special staining methods are difficult to apply to these as to other insect eggs. Apparently mitotic figures are normal now as well as in embryonic stages and I am induced to regard Buchner's figure of a multipolar spindle as an exceptional case. That such may occur I have no reason for denying, and it is indeed strange that abnormalities are not the rule rather than the exception in all of the mycetocytes.

The size of the cells is not proportionate to the increased amount of chromatinic material. It is augmented considerably when compared with that of the giant cells in the time of first association with the symbionts, but never reaches the dimensions that one would expect from an examination of the contained chromosomes.

General Considerations.

Breest's and Strindberg's conclusions that mycetocytes arise from the yolk cells may safely be discarded as far as *Pseudococcus* is concerned. By Strindberg's own definition, yolk cells are cleavage cells which have been left behind after the general migration to the periphery to establish the blastoderm. The giant cells however, which are the direct progenitors of the mycetoctes, arise even before the migration of cleavage cells is complete, and therefore before the yolk cells have been established as such.

Pierantoni's conclusions have already been taken up in my previous paper ('23). Undoubtedly he is correct in his explanation that the cleavage cells wander in among the symbionts, but in *Pseudococcus* I consider this association, if such it can be called, as one of the most temporary nature. It is the natural consequence of the general peripheral migration of cleavage cells.

The whole series of developments, as here described, seems extraordinary. And yet, most of the stages regarded individually are not unprecedented; the processes involved have been described before in other forms.

A fusion of polar bodies and the persistence of the polar nucleus thus formed has been described in several polyembryonic Hymenoptera. The formation of a polar nucleus is therefore not the only case among insects. It is indeed only rarely that such instances are met, but the old assumption that polar bodies never develop in a normal case of embryology certainly does not hold. That the polar nucleus does not behave like the female pronucleus is of course quite evident. In *Pseudococcus* for instance, its derivatives tend to stay at the periphery and do not sink into the egg as does the pronucleus. Nevertheless those inherent qualities in the latter which cause it to fuse with the male pronucleus, may be present to a certain extent also in the polar nucleus derivatives and cause them to combine with any cell that happens to come in contact with them. Certainly this tendency is not to be observed in the cleavage cells, for these are never found to fuse with each other under ordinary circumstances. It is found only in the polar nucleus derivatives, which can fuse both with each other and with the cleavage cells.

In much of their further behavior they are not anomalous at all. It is almost unnecessary to mention that in case of a great many animals and plants, complete fusion of the pronuclei may be delayed for some time. In such extreme cases as *Cyclops* (Rückert, '95; Haecker, '95) and *Cryptobranchus* (Smith, '19) the individuality of the two pronuclei may be traced even through the early cleavage stages. The failure of immediate fusion of two apposed nuclei in *Pseudococcus* is therefore not peculiar. As a matter of fact, it seems to be a rule in insects that the two pronuclei lie in apposition and the chromosomes of each are evolved independently of those of the other. It is only when nearly fully condensed that the nuclear walls break down and the chromosomes intermingle. Such seems to be the case in *Archimerus* (Morrill, '10), "goumi aphid" (Stevens, '06), *Trialeurodes* (Schrader, '20) and finally in the pronuclei of *Pseudococcus* itself.

Another aspect in the process of fusion of polar nucleus derivatives and cleavage cells is to be observed in the fact that two apposed nuclei may be in different phases. It has been explained that at such times the chromosomes of the nucleus in a more advanced phase are fully condensed but then enter on a period of suspended activity. During this period the chromosomes of the apposed nucleus or nuclei also become condensed and only then the common spindle is formed and the different sets of chromosomes are arranged in a single plate. The mechanism involved in this regulative process is not clear to me. But it may be stated that this aspect also is parallelled by the behavior of pronuclei in several forms. Here may be mentioned *Lilium* (Weniger, '18), the "goumi aphid" (Stevens, '06), and once more the pronuclei of *Pseudococcus* itself. In the latter case I have mentioned ('23) the possibility that delay in the condensation of chromosomes in one of the two pronuclei may be connected with the peculiar chromosome conditions of the male. This is at best only a working hypothesis.

The present account makes it evident that, generally speaking, the fusion of the polar nucleus derivatives with migrating cleavage cells is a more frequent occurrence than I had previously supposed. It therefore seems best to apply the name "polar nucleus derivative" only to the cells carrying 15 chromosomes, which are products of the division of the original, single polar nucleus. In my previous paper ('23) this term was applied somewhat indiscriminately to cells arising from division of the polar nucleus as well to some of those that had already undergone fusion with other cells and therefore contained a multiple number of chromosomes. The latter type of cell has been called "giant cell" throughout the present paper and of course includes cells arising from the fusion of polar nucleus derivatives among themselves, as well as with cleavage cells. The distinction between the giant cells and the polar nucleus derivatives is thus made a very definite one. Giant cells that have entered into association with the symbionts therewith become mycetocytes.

No attempt has been made here to discuss the exact relations between the insect body and the symbionts harbored by it. It should be pointed out however that the mycetocytes are insect cells. But they are in a measure extraneous to the organization of the body of the insect and even their actual connections with the latter are confined to branches of the trachea. Even during development they do not stand in a more direct relation to the various organs of the embryo than do the symbionts themselves. Their physiological importance nevertheless may be considerable; but this is a problem in itself.

I realize that the difficulties of the case are not removed by listing parallel instances of various stages—as I have done in this discussion. Such a proceeding however does serve to emphasize that many of the questions brought up by the investigation are identical with problems that have troubled the cytologist for years.

SUMMARY.

1. The first and second polar bodies of *Pseudococcus* undergo fusion and form a polar nucleus. This contains 15 chromosomes.

2. The polar nucleus divides several times (probably a definite number of times) giving rise to the polar derivatives.

3. The polar derivatives may fuse with either migrating cleavage cells or with each other to form the giant cells. The numerical data furnished by chromosome counts as well as the purely cytological evidence support each other in arriving at this conclusion.

4. The giant cells migrate from the periphery to the symbionts to enter into association with these. When this process has been completed, the giant cells are known as mycetocytes.

5. Discussion regarding the nomenclature of the cells involved in these phenomena. Statement of the problems presented during the various stages of the investigation.

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EXPLANATION OF PLATES

All figures have been drawn at table level, using a camera-lucida. Tube length = 160 mm., 2 mm. Zeiss oil immersion objective, and No. 12 X compensating ocular. No reduction has been made in the reproductions.

PLATE I. (all figures of *Pseudococcus citri*).

FIG. 1. Chromosomes of the fertilization nucleus.

FIG. 2. Blastoderm cell during early stage in the blastoderm formation.

FIG. 3. Blastoderm cell at time when blastoderm has become complete and giant cells have begun to migrate to the symbionts.

FIG. 4. The polar nucleus.

FIG. 5. One of two daughter cells derived from the first division of the polar nucleus, *i.e.*, one of first polar nucleus derivatives.

FIG. 6. One of two daughter cells derived from the division of one of the first polar nucleus derivatives. Four derivatives present in the egg at this time.

FIG. 7. Telophase of the third division of the polar nucleus. Eight derivatives in the egg when this division is complete.

FIG. 7*a*. One of four polar nucleus derivatives with fifteen chromosomes almost condensed for the next division. Cleavage cell in resting stage coming into apposition.

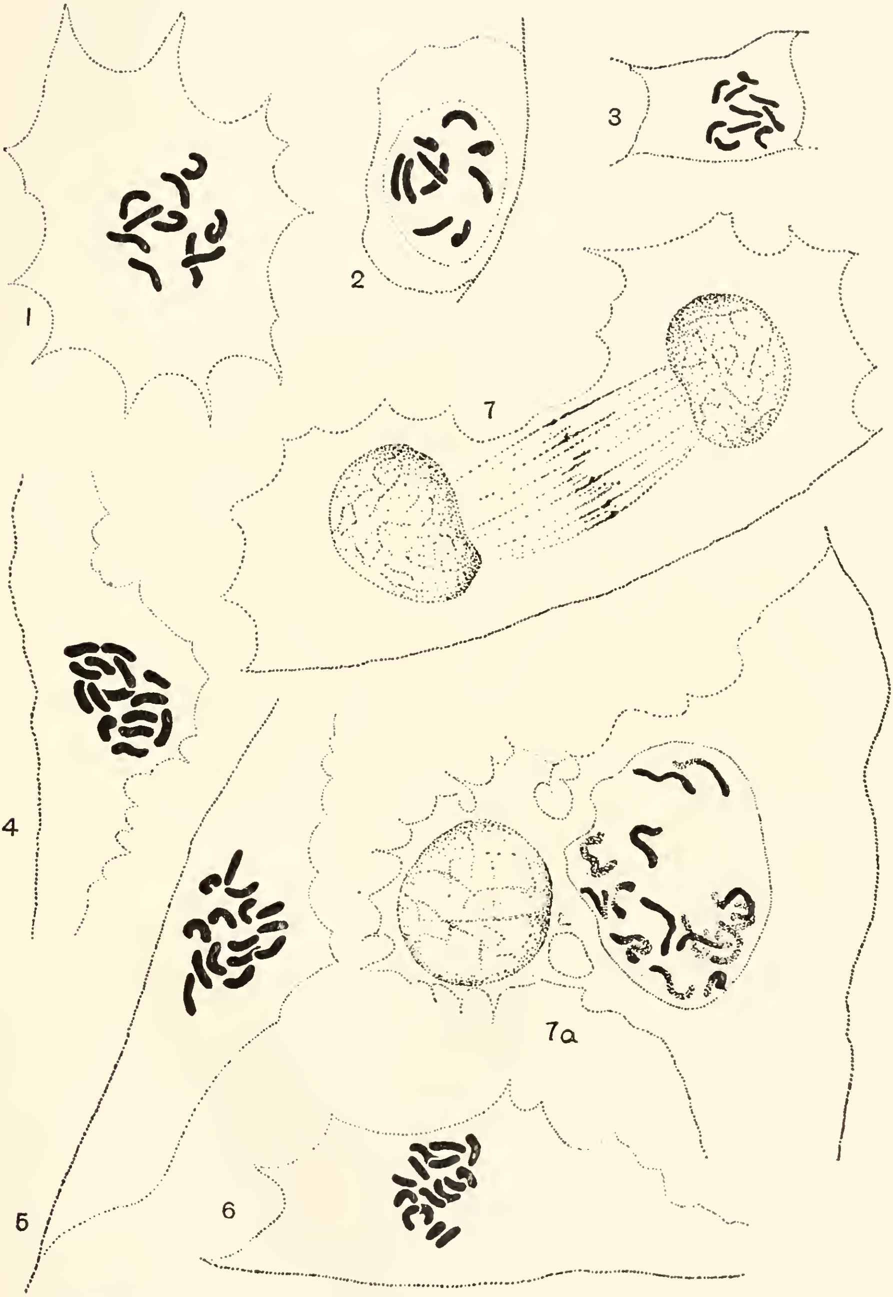

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PLATE II.

FIG. 8. Cleavage nucleus approaching a polar nucleus derivative. Cytoplasmic area fusing. (*P. maritimus.*)

FIG. 9. Two nuclei in apposition. Not certain whether both are polar nucleus derivatives. (*P. citri.*)

FIG. 10. Three nuclei in apposition. The two smaller with chromatin slightly more condensed than that of the larger nucleus. Probably two cleavage nuclei and a polar nucleus derivative. (*P. maritimus.*)

FIG. 11. Nucleus showing 20 chromosomes almost fully condensed and 15 at a slightly earlier phase. The 20 condensed chromsomes seem to be arranged in two groups of 10 chromosomes each. Probably originated from two cleavage nuclei and a polar nucleus derivative. (*P. maritimus.*)

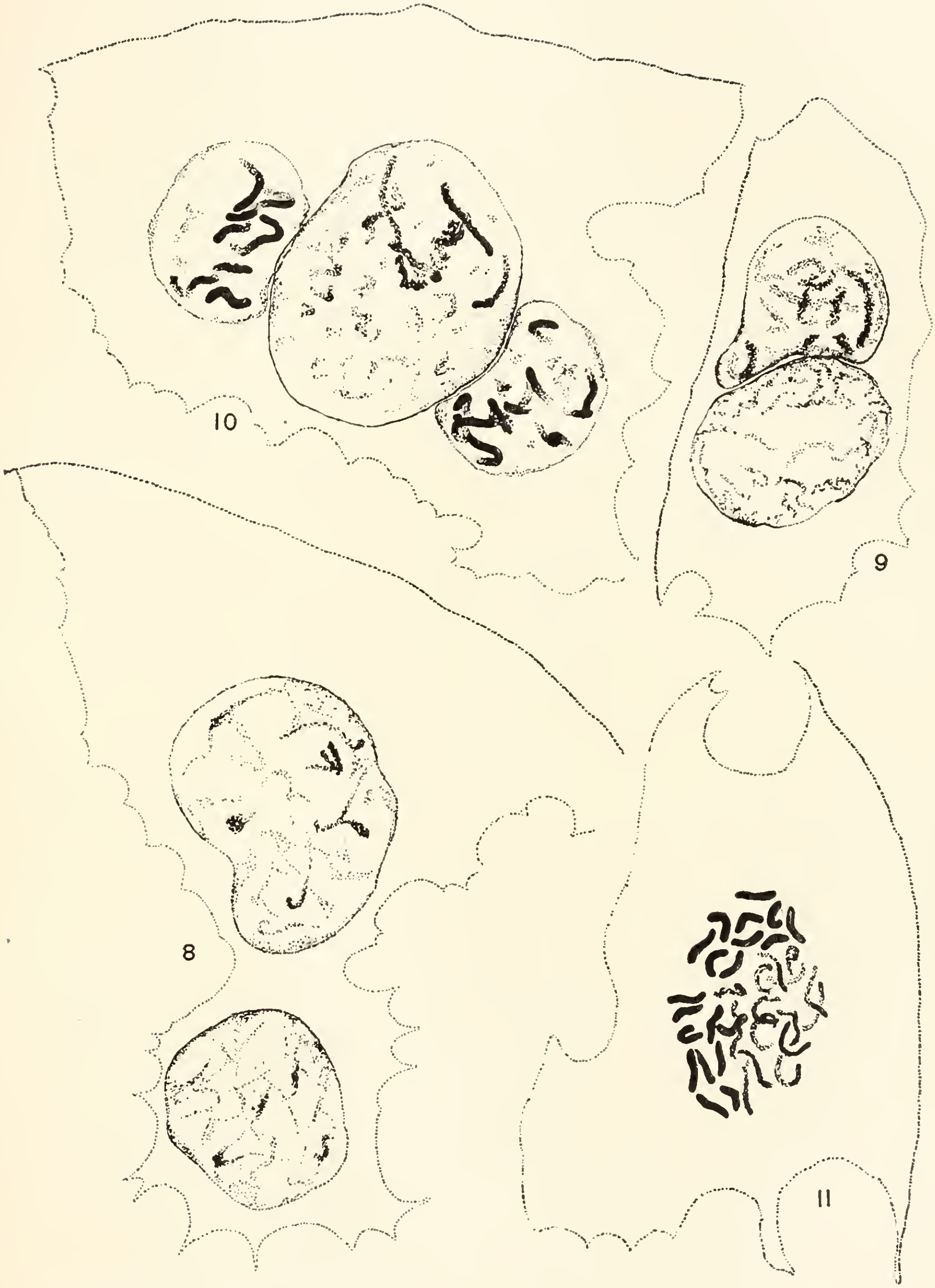

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Plate III.

Fig. 12. Giant cell containing 25 chromosomes. (*P. citri.*)

Fig. 13. Giant cell containing 25 chromosomes. (*P. citri.*)

Fig. 14. Giant cell containing 30 chromosomes. (*P. citri.*)

Fig. 15. Giant cell containing 25 chromosomes. (*P. maritimus.*)

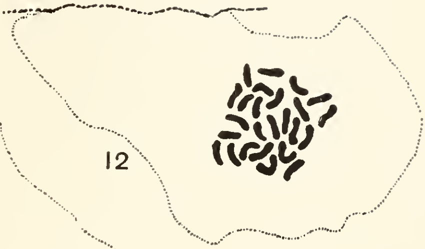
Fig. 16. Giant cell containing 35 chromosomes. (*P. maritimus.*)

Fig. 17. Giant cell containing 35 chromosomes. (*P. maritimus.*)

Fig. 18. Giant cell containing 60 chromosomes. (*P. citri.*)

Fig. 19. Mitotic division in a mycetocyte of an adult female. (Two other sections not shown.) (*P. maritimus.*)

Fig. 20. Metaphase plate of chromosomes in a mycetocyte of an adult female. (Two other sections not shown.) (*P. maritimus.*)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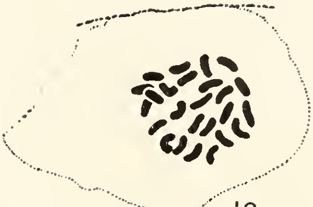
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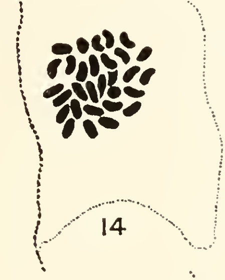
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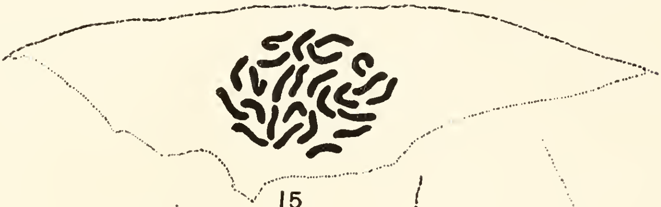
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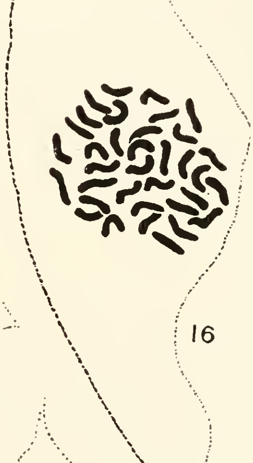
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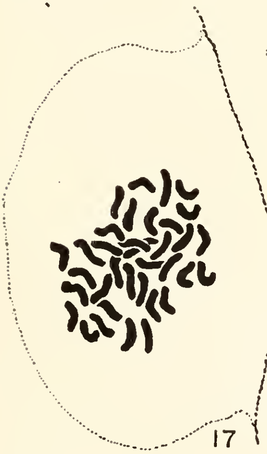
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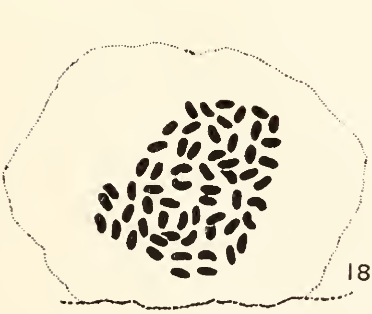
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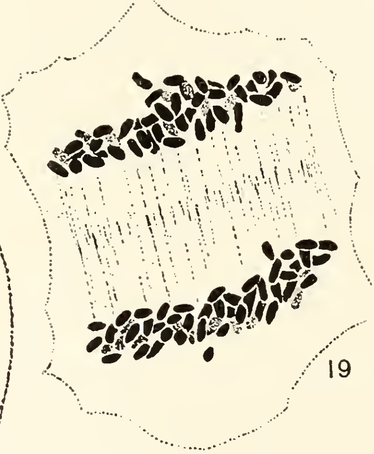
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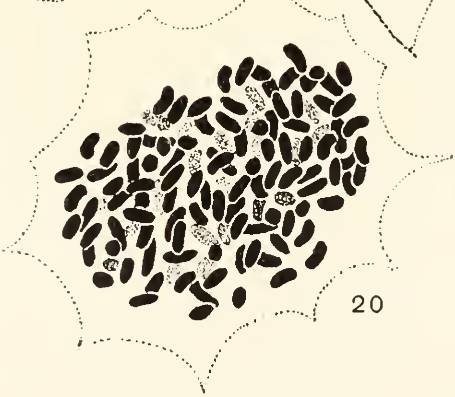
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THE ENDOCRINE SYSTEM OF *TYPHLOMOLGE RATHBUNI.*

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The endocrine system of *Typhlomolge rathbuni*, the blind Texan cave salamander, has been a matter of controversy for some time. In order to clarify some of the points under discussion, I have made serial sections of the region of the lower jaw, throat, and heart of 5 specimens, and the entire head of 2 specimens of this animal. The specimens were captured in Texas, as described in a previous article.[1] Six of them died during the trip from Texas to New York, one after 14 months of captivity in the laboratory. Since they are preserved after death, only the anatomical features of the various organs can be studied. A histological study must be postponed until suitable material can be secured.

THE THYROID.

Emerson[2] was the first one to call attention to the possible absence of the thyroid gland in *Typhlomolge*. In 1905 she examined sections through the head of one specimen and was unable to find a thyroid. At the time Emerson published her paper the interest in the endocrine system of amphibians was very slight and her paper remained unknown to most biologists. In several of my papers on the thyroid function of salamanders I have called attention to Miss Emerson's interesting findings, which I had recognized to be correct. Soon after my return from Texas in 1916, I sectioned one of the *Typhlomolge* captured there and found the thyroid absent.

But at the 1921 Christmas meeting of the Anatomists, Swingle, apparently unacquainted with the literature on these facts spoke of the thyroid of *Typhlomolge* as a matter of fact and claimed to have isolated and observed this organ under the microscope.

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[1] Uhlenhuth, E., *Biol. Bull.*, 1921. XL., 73.

[2] Emerson, E. T., *Proc. Soc. Nat. History, Boston*, 1905, XXXII., 43.

Although I mentioned my own findings, Swingle's very definite claims made the correctness of my observations doubtful, and even Doctor Wilder, from whose laboratory Emerson's paper was published, was ready to admit the possibility of an oversight on the part of Miss Emerson.

Immediately after my return from this meeting, I made sections of the 7 specimens discussed in this paper, and upon examination of the first one I was convinced that I was correct. In response to a letter in which Swingle admitted that the organ which he had claimed to be a thyroid was another vesicular organ, I communicated my new observations to Mr. Swingle. Neither this communication nor the incident at the Anatomists' meeting has been mentioned in an account recently published by Mr. Swingle in which he[1] states that 3 specimens examined by him possessed no thyroid.

The examination of the 7 specimens, together with previous findings, shows that while some specimens of *Typhlomolge* are without even vestiges of the thyroid, others possess epithelial structures, evidently undifferentiated thyroid rudiments whose development was inhibited by an unknown factor.

Before describing these rudiments, the location of a normal thyroid may be briefly referred to. For comparison I shall use the thyroid of the Ambystomidæ, which may be called representative of a normal salamander thyroid. In the Ambystomidæ, the median thryroid rudiment splits up into two epithelial cell masses, which migrate in a posterior, lateral and largely ventral direction until, in *Ambystoma opacum*, they are closely attached to a large lymphatic space (Fig. 1). This space is located in the interstitial space formed by the muscles which surround the first gill arch, ventral and median to the epibranchial of the first gill arch. In other species the thyroids may be located slightly further posterior, but always the lymphatic space where it is in touch with the thyroid is adjacent also to the anterior cardinal veins. At the site where the ventral end of the thyroid is attached to the lymph space, the anterior cardinal vein comes into close contact with this space, leaving there the thyroid, at its ventral and posterior end, as a large vessel into which collects the blood from the interfollicular rete of the thyroid.

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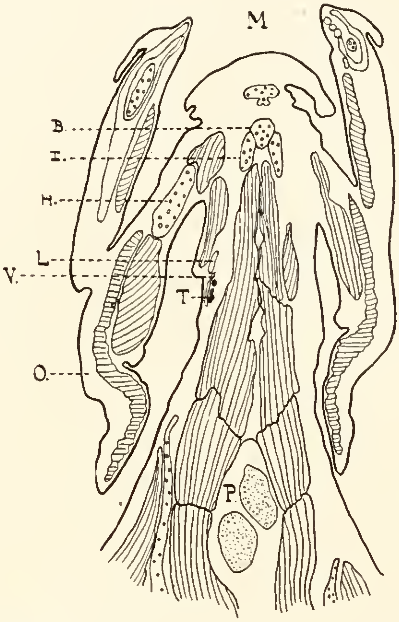
[1] Swingle, W. W., *Jour. Exp. Zoöl.*, 1922, XXXVI., 397.

In addition to the main portions of the thyroid, most specimens possess accessory thyroids. These develop from small cell groups which during migration become detached from the main portions and thus mark the path along which the main portions migrate. Their location varies greatly. They are located usually anterior and may be either ventral or dorsal or, in case of several accessories, both ventral and dorsal to the main portions. Or they may be at one level with the main portions. They consist either of one or several median rudiments located in the median interstitium of the muscles ventral to the basibranchials of the visceral skeleton (genio-hyoideus) or of two lateral portions, one on each side, which either are attached to the sides of the basibranchials or are located in the interstitia of the muscles lateral and ventral to the basibranchials. In some Ambystomidæ



In reproduction figures 1 to 8 have been reduced by one third, figures 9 and 10 by slightly more than one third.

FIG. 1. Location of main portion of right thyroid in an advanced larva (59.2 mm. total length) of *Ambystoma opacum; I*, first gill arch; *B*, first basibranchial; *H*, hyoid; *L*, lymph space; *M*, cavity of mouth; *O*, operculum; *P*, pericard; *T*, thyroid; *V*, anterior cardinal vein.

(*A. tigrinum*) the lateral portions may develop into normal thyroids of considerable size.

The thyroid rudiments of *Typhlomolge* occupy a position closely resembling the location of the various thyroid portions of the Ambystomidæ.

Typhlomolge 1, a sex-mature animal of 111 mm. total length and 58.2 mm. body length, does not possess even vestiges of a thyroid. The region of the lower jaw, throat, and heart was sectioned into a complete series; no section is missing. The anterior cardinal vein was followed in its entire course, the visceral cartilages and muscles were carefully searched through, but no traces of a thyroid could be found.

Typhlomolge 3, a small, apparently young, animal of 57.7 mm. total length and 32.6 mm. body length possesses a median thyroid rudiment. It is entirely detached from the pharyngeal epithelium and partly imbedded into the muscles just ventral to the basibranchial (genio-hyoideus). It is located between the attachments, to the basibranchial, of the hyoids and first gill arches and just anterior to the latter ones. The tissues are not well preserved, but the rudiment is seen to consist of several vesicles possessing epithelial walls and containing no colloid. No other epithelial structures were found, although the anterior cardinal vein was searched in its entire course down to the *Ductus cuvieri*, and the lymphatic space, the muscles, and cartilages of the visceral skeleton were carefully inspected.

In *Typhlomolge* 7, an animal of 75. 6 mm. total length and 43.1 mm. body length, a median rudiment is attached to the ventral surface of the muscles just ventral to the basibranchial, about in the middle between the attachments of the hyoid and first gill arches (Fig. 2). It consists of a single solid cell mass of epithelial structure (Fig. 3). In the center a network-like structure is noticed, produced very likely by the cell walls of the clear inner cell ends; the same structure is frequently found in tangential sections through the walls of the follicles of normal thyroids. No colloid is contained in this rudiment. In addition to the median rudiment, two lateral rudiments are present, one on each side. The anterior ends of these rudiments are located near the connection between ceratobranchial and epibranchial of the first gill arch and median to this arch (Fig. 4). They extend in a posterior direction; the posterior end approaches closely the wall of the lymph space, but does not come in contact with it. It is evident from this account that the lateral rudiments of *Typhlomolge* occupy a location similar to that of the main portions of the thyroid in *Ambystoma opacum*. They are, however, located further anterior, as if they had stopped migrating before attaining the definite position. Moreover, the place where the anterior cardinal vein passes the lymph space is located considerably more ventral; therefore, the lateral rudiments are nowhere in contact with this vein. It is indeed impossible to see any vessels supplying the thyroid rudiment;

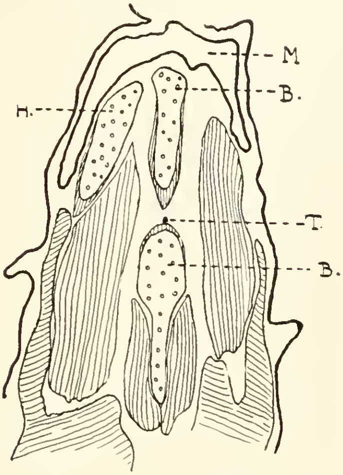


FIG. 2. Location of median thyroid rudiment in *Typhlomolge* 7. *B*, basibranchial; *H*, hyoid; *M*, cavity of mouth; *T*, thyroid rudiment.

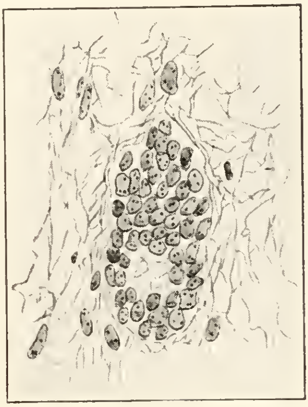


FIG. 3. Median thyroid rudiment of *Typhlomolge* 7. × 340.

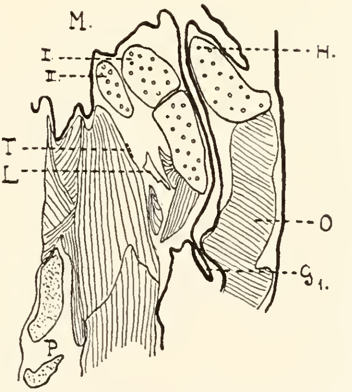


FIG. 4. Location of lateral thyroid rudiment of *Typhlomolge* 7. I., first gill arch; II., second gill arch; G_1, plate of first gill; *H*, hyoid; *L*, lymph space; *M*, cavity of mouth; *O*, operculum; *T*, thyroid rudiment.

if there are any they must be very small. The lateral rudiments consist of a series of tiny epithelial cell masses; some of them are solid, others are hollow, but none of them contain colloid.

In *Typhlomolge* 6, the smallest and probably youngest animal (56.0 mm. total length and 32.0 mm. body length), no median but one lateral rudiment on each side is present. They consist of a series of cell masses (Fig. 5), some of which are solid while others contain a lumen. Colloid is absent in all of them. The location is similar to that of the lateral rudiments of the previous specimen. In particular they do not touch the lymph space and are situated dorsal to the place where the anterior cardinal vein comes into contact with this space. They are without a blood supply resembling that of a thyroid.

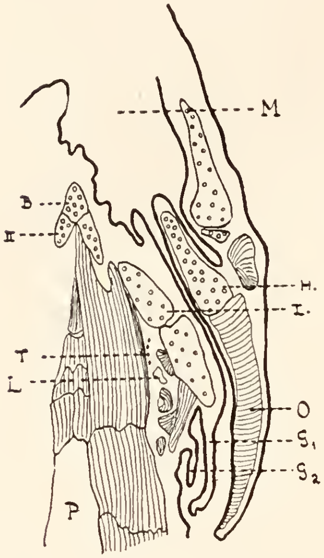


Fig. 5. Location of lateral thyroid rudiment of *Typhlomolge* 6. I., first gill arch; II., second gill arch; *B*, basibranchial; *G*₁, blade of first gill; *G*₂, blade of second gill; *H*, hyoid; *L*, lymph space; *M*, cavity of mouth; *O*, operculum; *P*, pericardium; *T*, thyroid.

In *Typhlomolge* 2, an animal of 77.5 mm. total length and 43.6 mm. body length, only the lateral rudiments are present. Their location is the same as that of the lateral rudiments described above. Instead of being broken up into several separate cell masses, each of them has the shape of one continuous epithelial cell tube possessing a narrow lumen (Fig. 6).

In *Typhlomolge* 5, an animal of 66.0 mm. total length and 36.5 mm. body length, only one lateral rudiment, the left one, is present. It is located near the connection between the ceratobranchial and epibranchial of the first gill arch, median to it and anterior to the location of a normal thyroid of *Ambystoma opacum*. It is composed of a small number of vesicles (Fig. 7) which, instead of being arranged in an antero-posterior row as in the other specimens, are crowded together in one place. The vesicles have a distinct epithelial lining and are hollow; they do not contain colloid. In addition to this rudiment, *Typhlomolge* 5 possesses another one of similar structure, located on the same side but more median. It is attached to the left side of the muscle just ventral to the basibranchial and just anterior to where the first gill arch connects with the basibranchial. Thus it has very nearly the same location as the median rudiment of other animals, but is displaced slightly to the left side. Apparently the primary median rudiment of this animal split into two rudiments; one of them, the left one, moved into its normal position. The right one not only failed to do so, but was dragged along a short distance by the left rudiment before complete separation was

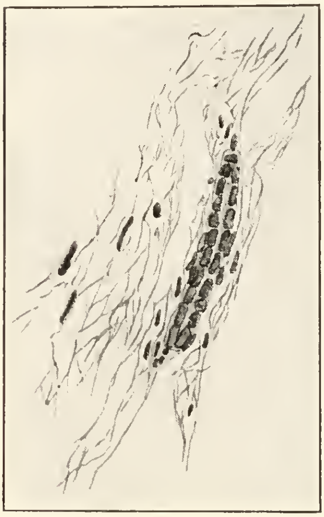


Fig. 6. Left lateral thyroid rudiment of *Typhlomolge* 2. × 340.

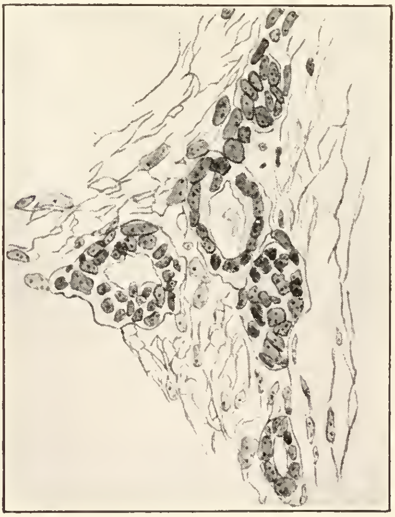


Fig. 7. Left thyroid rudiment of *Typhlomolge* 5. × 340.

accomplished, and thus was dislocated from its primary median position to wards the left side.

The 6 specimens described so far died on the way from Texas to New York, shortly after they had been removed from the caves. The seventh animal, *Typhlomolge* I, a specimen of 97.5 mm., died after it had been kept alive in the laboratory for 14 months. The largest part of this time (12 months) it lived at a temperature of 15° C. and in darkness; for the last two months it was kept in an aquarium stocked with plants, small crustaceans and young tadpoles, in daylight and at a temperature of approximately 22 to 25° C. The sections of this specimen are greatly torn and I am not sure that our inability to find a median and left lateral rudiment is due to the absence of these organs and not to the poor condition of the sections. On the right side, however, a lateral rudiment is present. It is located near the lymph space, anterior and dorsal to the location of the main portion of the thyroid of *Ambystoma opacum* and resembles more closely a thyroid structure than the rudiments of the specimens described previously. It consists of a small number of hollow vesicles which compose an elongate, egg-shaped organ possessing a connective tissue capsule and hence impressing one as a distinct and individual organ. The walls of the vesicles are of epithelial character; but no colloid is contained in the lumen of the vesicles (Fig. 8).

Summary: Although *Typhlomolge*, in its advanced stages, does not possess an organ resembling the normal thyroid of a salamander, epithelial structures are found which indicate that in the young embryo of this animal the thyroid rudiment forms in a similar manner as in other amphibians. This rudiment, however, for some reason, fails to develop into a thyroid. In some animals, development ceases after the median epithelial outgrowth has separated from the pharyngeal epithelium and the rudiment remains a single vesicle. In other cases it may partly split up into lateral portions which, as in other salamanders, move in a posterior direction and, in some instances, may approach closely the lymph space; but they never reach the anterior cardinal vein. The unsplit part of the median rudiment may retain its median position and primitive vesicular structure; the lateral portions may either develop into a continuous epithelial tube or may break up into a series of solid or hollow cell masses. In some animals no median rudiment is found; if this condition develops in consequence of a complete splitting-up of the median rudiment or of later degeneration of the median rudiment, can be decided only by studying the embryology of this animal. In one specimen no vestiges of the thyroid are present at all. If Emerson's and Swingle's statements are not due to an oversight, on their part, of the inconspicuous epithelial structures, there are now 6 specimens of *Typhlomolge* known which did not possess any vestiges of the thyroid. One was described by Emerson, 3 by Swingle, one was found previously by me and the sixth animal is the one described in this paper. Either no thyroid rudiments developed in these six animals, or they were reabsorbed shortly after they had developed.

It is certain that the thyroid rudiments of *Typhlomolge* which do persist retain permanently a primitive epithelial structure and fail to develop, among many other structures of a normal thyroid, a venous rete and the colloid.

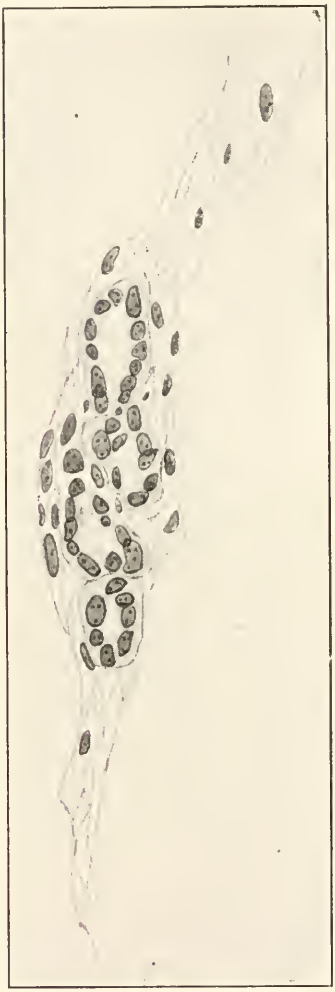


FIG. 8. Right thyroid rudiment of *Typhlomolge* I. ×. 340.

OTHER ENDOCRINE ORGANS.

It may be briefly mentioned that the thymus glands, the hypophysis, and the postbranchial body were found to be present in every specimen. Like other salamanders, *Typhlomolge* possesses 3 pairs of thymus glands; in one animal they were found fused into two large glands, one on each side. This condition is frequently met with in adult salamanders.

The postbranchial body, although, on a whole, it resembles this organ in other salamanders, shows certain peculiarities (see Baldwin's paper [1] for a description of this organ). Its structure is very similar to that found in *A. opacum;* in particular, it is found only on the left side. It is an epithelial structure of the shape of a tube possessing, in places, epithelial diverticula. A lumen is frequently absent, while in *A. opacum* and other Ambystomidæ this organ possesses often a very condiserable lumen. The cephalic end of the organ is located in the pharyngeal epithelium with which it connects near the place where in Ambystomidæ the *Aditus laryngeus* is situated. In the Ambystomidæ the posterior end of the organ is often very large as compared to the thin duct-like anterior end and is located on the left side of the pericardium, posterior to the fourth aortic arch. Frequently it is closely attached to the pericardium and posterior wall of the fourth aortic arch. In *Typhlomolge* the fourth aortic arch is missing; the postbranchial body attaches itself to the third aortic arch. In some animals it reaches back to the heart and is found attached to the pericardium. Its posterior end, however, does not attain the size which this part is found to attain in *Ambystoma.* Moreover, in some specimens the organ remains short, extending backward only to the middle between pharynx and pericardium. In these cases its posterior end becomes attached to the wall of the third gill arch approximately half-way between the pericardium and the entrance of the arch into the gill blade of the third gill. It seems that the postbranchial body of *Typhlomolge,* although it possesses, on the whole, the structure of the normal organ of the Ambystomidæ, shows sometimes signs of developmental inhibition.

The hypophysis was studied only in two animals and only in transverse sections. Like the hypophysis of the Ambystomidæ [2] it is composed of 4 parts, the pars anterior proper, the partes tuberales, the pars intermedia and the pars nervosa. In the pars anterior, the largest part of the entire organ, the individual tubes are discernible more distinctly than in the Ambystomidæ. They take an antero-posterior course and are arranged parallel to each other (Fig. 9). In the spaces separating the individual tubes, large blood vessels are located. Frequently the individual tubes possess a distinct lumen; the nuclei of the cells are located at the distal end of the cell, towards the lumen of the tube. The cell walls are sometimes very distinct. The anterior end of the pars anterior continues into two lateral processes, the partes tuberales,

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[1] Baldwin, F. M., *Jour. Morph.*, 1918,XXX., 605.

[2] Atwell, W. J., *Anat. Record*, 1921, XXII., 373.

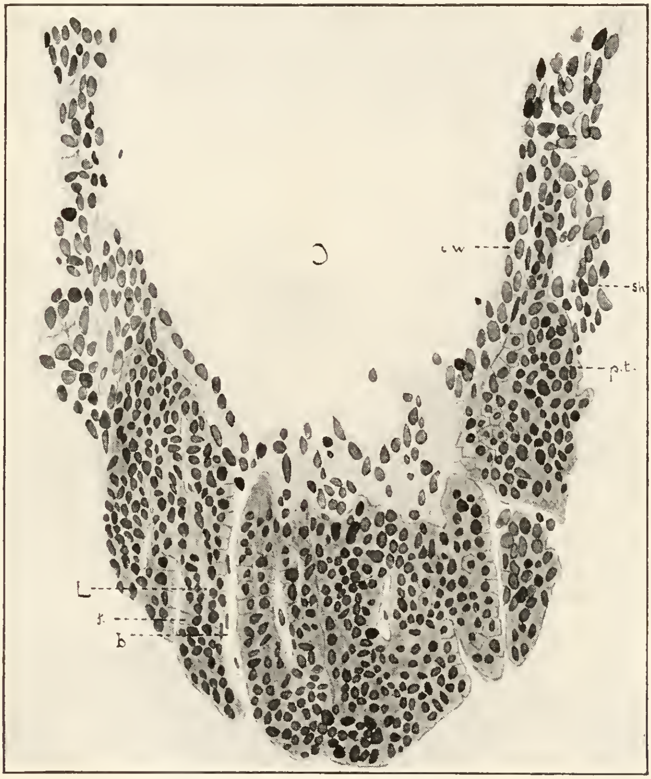

Fig. 9. Transverse section through the hypophysis of *Typhlomolge* 7. *b*, blood vessel; Ɔ-infundibulum; *i.w.*, infundibular wall; *L*, lumen of individual tube; *p.t.*, left pars tuberalis (of the right pars tuberalis this section contains only the connective tissue sheet of the ventral surface). *Sh*, connective tissue sheet. *t*, individual tube of pars anterior. × 340.

which, like in other salamanders [5, 6, 7], are continuous with the pars anterior and attach themselves to the ventral wall of the infundibulum (Fig. 9). The partes tuberales of *Typhlomolge* are apparently smaller than in the adult *A. opacum* and resemble in size the partes tuberales of a larvæ of *Ambystoma opacum* of about 55 mm. total length and showing no signs of metamorphosis as yet. The pars intermedia of the amphibians cannot well be discriminated from the anterior part in transverse sections. But from such sections as shown in Fig. 10, it would seem that the dorsal part of the pars intermedia is bilobed, the two lobes being separated by a median antero-posterior space. The pars nervosa, as in other salamanders, consists in a thickening of the wall of the infundibulum where the pars intermedia is attached to it (Fig. 10). In comparing the pars nervosa of *Typhlomolge* with that of other salamanders, Haller's description [7] of the pars nervosa of *Proteus anguineus* is of interest. According to this author, the pars nervosa of Proteus is hardly differentiated from the rest of the infundibular wall. In *Typhlomolge* the pars nervosa is not only well-differentiated, but seems to be larger and more sacculated than is the case in *Ambystoma opacum* (Fig. 10). Summarizing the description of the hypophysis of *Typhlomolge*, one may say that it resembles closely the hypophysis of other salamanders. In particular, it does not seem that the hypophysis of this species presents indications of an atrophic state, although, with a larger amount of material at our disposal we might find that the partes tuberales of *Typhlomolge* are of the size of a larval organ.

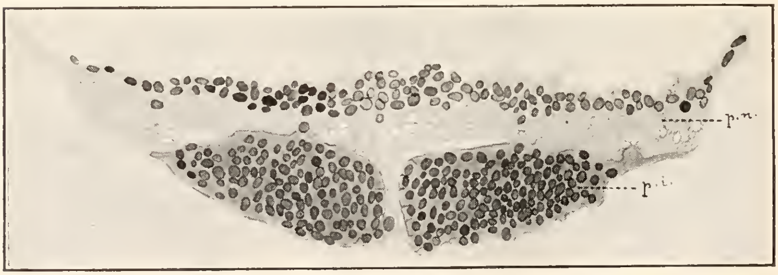

FIG. 10. Transverse section through dorsal regions of hypophysis of *Typhlomolge* 7. *p.i.*, pars intermedia; *p.n.*, pars nervosa. × 340.

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[6] Haller, B., *Morph. Jahrb.*, 1898, XXV., 30.

[7] Haller, B., *Arch. Mikr. Anat. und Entwicklgsg.*, 1909, LXXIV., 812.

DISCUSSION.

The factor which led to the inhibition of the thyroid development is unknown. The researches of Leo Adler [8] and Bennett M. Allen [9] showed that extirpation of the hypophysis in amphibians inhibits the development of the thyroid. One could imagine that defective development of the hypophysis might have been the immediate cause of the inhibition of the thyroid development in *Typhlomolge*, but so far, no abnormalities in the structure or mutual relations of the various parts of the hypophysis have been discovered, which could account for the thyroid atrophy of *Typhlomolge*—a phenomenon singular in the vertebrates.

It is not known whether the atrophy of the thyroid of *Typhlomolge* is only one of the results caused by certain factors which lead to the inhibition of the general development of this animal, or whether the inhibition of the thyroid was primary to other developmental inhibitions. As pointed out in previous papers [10] the athyroidism of this species possesses special interest, because in the same species metamorphosis also is suppressed. I have assumed, in a purely tentative manner and in order to obtain a basis for further experiments, that the latter phenomenon is the direct result of the lack of the thyroid. In the absence of adequate experiments and in the light of the well known fact that extirpation of the thyroid inhibits the amphibian metamorphosis [11], [12], this explanation still seems to be the most feasible one.

Swingle,[13] in a recently published article, takes occasion to criticise my attitude, outlined above, towards the problem of neoteny in *Typhlomolge*. He has made certain observations confirming the existence of a releasing mechanism in salamanders. Regarding the facts communicated in this article, these surely should be welcome to the writer of the present article, in so far as they led Swingle to exactly the same conclusion as that at which I arrived as early as 1919. But certain statements made in this paper are apt to give rise to misunderstandings. To prevent these a discussion of Swingle's paper seems desirable.

[8] Adler, L., *Arch. Entwcklgsmech. d. Org.*, 1914, XXXIX., 21.

[9] Allen, B. M., *Biol. Bull.*, 1917, XXXII., 117.

[10] Uhlenhuth, E., *Endocrinology*, 1922, VI., 102.

[11] Allen, B. M., *Science*, 1916, N. S. XLIV., 755.

[12] Hoskins, E. R., and Hoskins, M. M., *Jour. Exp. Zoöl.*, 1919, XXIX., 1.

[13] Swingle, W. W., *Jour. Exp. Zoöl.*, 1922, XXXVI., 397.

Throughout his paper Swingle attempts to create in the reader's mind the impression that in my previous work I have laid too much one-sided emphasis on the thyroid gland as the only organ potent in the amphibian metamorphosis. This attitude is perplexing in view of the circumstance that I have been able to disclose facts demonstrating the existence of a releasing mechanism outside of the thyroid and in view of the other circumstance that Swingle received the discovery of this "releasing mechanism" when communicated by the writer in 1919[14] in the following way[15] (p. 600): "Uhlenhuth, while accepting the conclusions stated regarding the relation of iodine to amphibian metamorphosis, thinks that still another substance is needed to cause the thyroid gland to excrete the iodine necessary for metamorphosis. This hypothetical factor he terms excretor substance and thinks that it is evolved during the growth processes of the organism. The assumption of an excretor substance obscures rather than clarifies the already sufficiently complicated problem of amphibian metamorphosis."

As to Swingle's criticism regarding the omission, on my part, of the possible defectiveness of the releasing mechanism in the explanation of the neoteny of *Typhlomolge*, it should be pointed out that the writer of this article has, reluctantly, refrained from suggesting this possibility, because no experiments suggesting it have been—or are today—available. As to the actual interpretation of the neoteny of *Typhlomolge* and as to my attitude[16] towards Jensen's[17] experiments which showed that adult *Proteus* and *Necturus* do not metamorphose upon thyroid administration, the following statements may be made: (1) the interpretation of this phenomenon as given in my previous papers[16] does not form an integral part of the theory of a releasing mechanism; (2) the most pertinent problem in regard to the neoteny of *Typhlomolge* was the question whether or not this animal possesses a thyroid gland proper, a question to the solution of which Swingle has contributed nothing, as will become evident from a perusal of the introduction to this article and of the facts described above; (3) the lack of an effective releasing mechanism may have been the primary cause of the neoteny of *Typhlomolge*, but so far nothing as to this effect can be quoted; (4) the question of whether or not *Typhlomolge* and phsyiologically similar species, such as *Proteus* and *Necturus*, possess, in their present state, the ability to metamorphose upon thyroid administration is a problem entirely aside from the rôle of the releasing mechanism and has been considered so in the writer's previous papers. (It is possible that permanent suppression of the thyroid function over long periods may cause complete loss of the reactivity of the organism. The demonstration of such complete loss, however, does not decide the question as to whether primarily the thyroid ceased to function, in *Typhlomolge*, on account of a defective releasing mechanism); (5) it has not been proved as yet that *Typhlomolge*, *Proteus*, and *Necturus* have completely lost their ability to react to the thyroid hormone. It was merely this point which gave occasion for the criticism of Jensen's work. Jensen [17] exposed only the adult specimens of *Proteus* and *Necturus* to the action of the thyroid hormone. That he did not succeed in enforcing metamorphosis does not necessarily mean that the responsiveness of these animals has been completely lost. In order to show that *Necturus*, *Proteus*, and *Typhlomolge* could not metamorphose even if they were in the possession of a complete and normal thyroid mechanism, the young larvæ or even the parents at the time of development or ripening of the ova and spermatozoa may have to be subjected to thyroid administration. Swingle has merely repeated Jensen's experiments on *Necturus*, without modifying Jensen's technique. Like Jensen, he did not use the young larvæ, but the adult animals. Nowhere in Swingle's paper, however, can there be found any reference to Jensen's experiments on *Necturus*.

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[14] Uhlenhuth, E., *Jour. Gen. Phys.*, 1919, I., 473.

[15] Swingle, W. W., *Jour. Gen. Phys.*, 1919, I., 593.

[16] Uhlenhuth, E., *American Naturalist*, 1921, LV., 193.

[17] Jensen, C. O. *Oversigt klg. Danske Vidensk. Forhandl.*, Copenhagen, 1916, No. 3, 251.

The same attitude is met with in Swingle's paper regarding the releasing mechanism. Although no progress beyond the present state of the problem has been accomplished, there is, in Swingle's article, no mention made anywhere of previous work on the same problem.

That the thyroid mechanism of salamanders consists of two physiologically distinct parts was found by the writer of this paper as early as 1919.[14] The discovery of the factor necessary to release the thryoid hormone was summed up in the following statement,[14] (p. 476): "Hence, besides iodine, still another substance is needed in the amphibian metamorphosis; namely the 'excretor substance' which causes the thyroid to excrete the stored-up iodine." Swingle's statement [15] (p. 600), made in 1919 in reference to this work and quoted above, shows that he did not recognize the existence of such a releasing factor.

Since organs of internal secretion or any other organs would manifest themselves physiologically in a manner essentially similar to a substance and since it seemed undesirable to reflect in the term applied to the releasing factor upon any preconceived theory, the term "excretor substance" was replaced later on by the term "releasing factor" [16] (p. 207) and "releasing mechanism" [10] (p. 112), both of them implying merely the function by which this factor manifests itself and which was actually observed.

Since the first communication was made my experiments were continued and it was shown, for 3 different species of salamanders, that in low temperature metamorphosis is greatly retarded in proportion to general growth, while the development of the thyroid gland shows no such retardation. It was concluded from this fact that since the thyroid developed at a normal rate in proportion to general growth the development of the releasing mechanism was retarded. Several papers were published in regard to this problem and the difference between the retardation of the thyroid and the releasing mechanism in response to the same degree of lowered temperature was explained by assuming a lower temperature coefficient for the thyroid than for the releasing mechanism. In 1921 the results of this work were summarized in the following statement [16] (p. 206): "The most conspicuous character in the salamander metamorphosis is the fact that although it certainly is dependent on the thyroid hormone, it does not necessarily take place in larvæ whose thyroid is mature. This can only mean that two factors are required in order to bring about the metamorphosis of salamander larvæ, namely a mature gland and a factor which releases the thyroid hormone from the follicles of the gland."

Further confirmation of the existence of a releasing mechanism has been found in the iodine experiments.[10, 18, 19, 20] Administration of an excess of inorganic iodine does not enforce the metamorphosis of salamander larvæ,[10, 20] yet the elaboration of the colloid is accelerated by iodine feeding. This result was to be expected if the release of the hormone does not depend on the quantity of hormone developed in the follicles of the thyroid but is controlled by a particular releasing mechanism.

The results outlined above were checked also by histological sections of large numbers of thyroids of normal and experimental animals. Although the publication in full of this work has been postponed in order to assure greater completeness, single results have been referred to in various papers and have been demonstrated to colleagues and before meetings. In every case it was found that the elaboraion of the colloid and the excretion of it are two distinct and independent processes, physiologically as well as structurally. Elaboration of normal colloid is frequently met with in cases of inhibition of metamorphosis and in normal larvæ long before metamorphosis, and, in this case, is combined with complete absence of the structures characteristic of the excreting stage of the thyroid. This relation has been interpreted as further testimony in favor of the existence of a releasing mechanism.

I must also refer here to Swingle's criticism of my iodine experiments, since, if correct, it would question the value of these experiments as supporting the theory of the releasing mechanism. My experiments [10, 20] showed that, contrary to anuran larvæ, in the larvæ of salamanders metamorphosis cannot be enforced by the administration of inorganic iodine. The bearing of this fact upon a general theory of the rôle of iodine in the specific effect of the thyroid hormone has been outlined in detail in two previous papers.[10, 20] Swingle's general attitude in his paper tends to create the impression (1) that I have claimed "iodine has nothing to do with the axolotl metamorphosis" [13] (p. 417) and (2) that somewhere in my papers are to be found statements to the effect that organic iodine compounds cannot enforce the metamorphosis of the axolotl and other salamanders.

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[18] Uhlenhuth, E., *Jour. Gen. Phys.*, 1922, IV., 319.

[19] Uhlenhuth, E., Biol. Bull., 1921, XLI., 307.

[20] Uhlenhuth, E., Biol. Bull., 1922, XLII., 143.

As to the first point I should like to refer the reader to the following statement,[10] (p. 114) into which my results on the rôle of iodine were summarized: "That iodine if supplied in excess does not produce metamorphosis of salamander larvæ does not mean, according to what has been said above, that it is not necessary in the metamorphosis of salamanders. Very likely if larvæ of salamanders would be raised on an iodine-free diet and kept in iodine-free water, metamorphosis could not take place."

Regarding the second point Swingle quotes against me his own experiments [13, 21] in which he thinks he has shown that 3–5 di-iodo-tyrosine can enforce metamorphosis of thyroidectomized axolotls, and Jensen's experiments (22) with iodized proteins. Neither Swingle's own experiments nor Jensen's experiments referred to have proved that inorganic iodine can be utilized directly by the axolotl tissues to elaborate the thyroid hormone. The facts regarding the influence of inorganic iodine on the axolotl metamorphosis are, however, widely different from what Swingle would like them to be.

In the first place, Jensen has not only not shown that inorganic iodine does enforce metamorphosis of the axolotl, but on the contrary has shown that inorganic iodine as such is ineffective in the axolotl metamorphosis. In one of his papers, Jensen [23] points out that the effectiveness of thyroid preparations in enforcing the axolotl metamorphosis does not correspond to the iodine-content of these preparations. In a personal conversation, Professor C. O. Jensen told me that he had tested the action of inorganic iodine, but found it ineffective in enforcing the axolotl metamorphosis. Jensen's experiments are therefore entirely in accord with my own experiments. Moreover, Professor Jensen's experiences which are well in accord with my own observations may serve as a warning against the reliability of those experiments which resulted in "enforced metamorphosis" of the axolotl. Among Professor Jensen's strains of the European race of the axolotl there were, in the beginning, animals which gave rise to offspring 50 per cent. of which would metamorphose

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[21] Swingle, W. W., *Science*, 1922, N. S., LVI., 720.

[22] Jensen, C. O., *Compt. rend. Soc. Biol.*, 1920, LXXXIII., 315; 1921, LXXXIV. 423; 1921, LXXXV., 391.

[23] Jensen, C. O., *Hospitalstidende*, 1920, LXIII., 505.

spontaneously. Early in his work he began to select carefully individuals which produced 100 per cent. neotenous larvæ.

Swingle also quotes the experiments of Huxley and Hogben,[24] and of Hirschler [25] against me. What Huxley and Hogben really found, however, is that inorganic iodine does not enforce the metamorphosis of axolotls. There are still Hirschler's experiments; these are represented by "one" successful experiment. The total number of Hirschler's experiments on inorganic iodine in relation to axolotl metamorphosis is "two." One animal was given an intraperitoneal injection of iodoform; it died before a conclusive result was obtained. The other animal received an injection of iodine dissolved in potassium iodid; it metamorphosed completely. But the animal illustrated, as a control alongside this experimental animal, shows, contrary to the authors claim, distinct signs of metamorphosis, a reduction of the tail fin and instead of the larval gills mere stubs. It seems to me the number of Hirschler's positive experiments will have to be increased before they can be held against the negative experiments of Jensen, Huxley and Hogben, and myself.

As to Huxley's and Hogben's positive results [24] on the larvæ of *Salamander maculosa* and *triton*, quoted by Swingle against me, it should be stated that the method employed in these experiments is such as to permit of no conclusions whatsoever. In the first place, they did not use the first moulting, but the sizes of the gills as an indicator of metamorphosis. The gills may become reduced in size by the action of many factors different from metamorphosis, particularly by starvation. Since strong iodine solutions were used, it is almost certain that contrary to the authors' impression (quantitative measurements of the food intake were not made) the experimental larvæ fed less well than the controls. Secondly, nowhere in Huxley's and Hogben's paper can I find any statement indicating the size and stage of the larvæ at the beginning of the experiment. Yet if the larvæ were in an advanced larval stage any irritation as serious as that caused by iodine solutions would be sure to bring about precocious metamorphosis.

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[24] Huxley, J. S., and Hogben, L. T., *Proc. Royal Soc.*, 1922, XCIII., 36.

[25] Hirschler, J., *Arch. Entwcklgsgsmech. d. Orgn.*, 1922, LI., 482.

It is evident that none of the observations according to which inorganic iodine does enforce the metamorphosis of salamanders can be accepted as correct at the present time.

That organic iodine compounds may enforce the metamorphosis of neotenous forms of salamanders has been claimed repeatedly and may be true, although the axolotl used generally in these experiments appears, for reasons stated above, to be an unreliable material. Jensen was the first one who studied, in an extensive manner, the influence of organic iodine-compounds upon the metamorphosis of axolotls. Where he left the problem it is still at the present time. In particular, Jensen deserves the credit for having recognized that the experiments with iodine could not advance the problem unless thyroidectomized larvæ are used. He was the first one who administered organic iodine compounds to thyroidectomized axolotls and stated [26] that thyroxine can be used directly by the organism without the intermediation of the thyroid. Swingle repeated these experiments [13, 21] using 3-5 di-iodo-tyrosine, a substance which Jensen [27] had found ineffective in the normal axolotl. Swingle reports that 3-5 di-iodo-tyrosine does enforce the metamorphosis of thyroidectomized axolotls. Both Jensen's and Swingle's experiments, however, should be taken with caution as far as the successful thyroidectomy is concerned. I am not certain at all that Swingle realizes that an axolotl possesses 4 thyroid glands, two main portions and two accessory ones. He mentions it nowhere and it is likely that only the main portions were extirpated. The accessory thyroid glands of *A. tigrinum* have a tendency to become very large and, after removal of the main portions, may enlarge considerably, so as to cause finally metamorphosis, as I observed in many larvæ of *A. tigrinum*. It is likely that Swingle's "thyroidless" axolotls were in the possession of two developing accessory glands; that an axolotl does not possess accessory glands I would be willing to believe only if sections through the entire region of the lower jaws, throat, and heart could be presented, since dissection, because of the hidden position of these accessory glands, may fail to demonstrate them. If the main thyroids are removed, it takes a long time before the accessories, in the event that they have been small, attain a size and structure capable of producing metamorphosis. But as shown in my iodine experiments, the feeding of iodine would greatly accelerate the elaboration of the hormone and, if the releasing mechanism is set active (which it was in Swingle's specimens, according to his own statements), metamorphosis may occur months before it takes place in the untreated controls. Swingle has observed his animals apparently only for 6 months; it would be important to know whether the untreated "thyroidectomized" animals did not finally metamorphose.

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[26] Jensen, C. O., *Compt. rend. Soc. Biol.*, 1921, LXXXV., 391.

[27] Jensen, C. O., *Compt. rend. Soc. Biol.*, 1920, LXXXIII., 315.

Swingle[13, 21] mentions also that 3–5 di-brom-tyrosine, when fed to thyroidectomized axolotl larvæ, is incapable of enforcing metamorphosis and thinks that this result is contrary to my own views on the rôle of iodine in the amphibian metamorphosis and in the thyroid hormone. Apparently he did not see the following statement, in which my views were summarized[10] (p. 114): "The views elaborated above are in no way contradictory to the fact that nevertheless, in a biological sense, iodine is an important and essential part of the thyroid hormone; if it were possible to substitute the iodine by any other substance without changing the reactivity of the hormone, biologically this would not make iodine less important, for it is the only substance which, by the mechanism actually available to the organisms, can be used in the manufacture of the thyroid hormone. Although chemically bromine or any other halogen may be able to substitute iodine without changing the chemical or even the physiological reactivity of the thyroid hormone, the organism is unable to use bromine, as shown by Swingle, and presumably the other halogens to make thyroid hormone." I have never claimed that the thyroid or any other organ can manufacture the thyroid hormone from bromine. Swingle has not touched, by his experiments, the real problem. This centers around the question whether the finished thyroid hormone could enforce metamorphosis if it contained bromine instead of iodine; Swingle did not employ such a product in his experiments.

As Swingle correctly states, the crux of the problem of thyroid function is now to find the organ or tissue or substance which plays the rôle of a releasing mechanism to the thyroid gland. I have intentionally refrained, in my previous papers, from forming any theories, aside from those directly suggested by the results of my experiments, as to the nature of the releasing mechanism; devoting pages to discussing assumptions and hypotheses does not materially advance the problem. We know, of course, that the hypophysis has something to do with the development and, possibly, with the function of the thyroid. I have made some experiments, to be published shortly, which seem to indicate that some unknown factor is located in the gills, in the absence of which the thryoid, although it develops in a normal manner, remains incapable of releasing the hormone. Swingle mentions one experiment which was intended to test the activity of the hypophysis of a neotenous axolotl by the grafting method. Although ultimately it may turn out that the hypophysis controls, in some way, the releasing mechanism, Swingle has so far contributed nothing to the solution of this problem.

Hence it is very evident that Swingle has not advanced, by a single step, the problem of neoteny and thyroid function beyond the stage at which my own researches left it.

SUMMARY.

1. Only in one, a sex-mature specimen, among 7 specimens of *Typhlomolge rathbuni*, is the thyroid completely absent; in the other 6 specimens rudiments of the thyroids are present.

2. The thyroid rudiments are undifferentiated epithelial cell masses located along the path of migration of the thyroid, typical for salamanders. They may contain a lumen, but never contain colloid and blood vessels.

3. *Typhlomolge* possesses 3 pairs of thymus glands.

4. The hypophysis is similar to that of other salamanders. But the partes tuberales are perhaps smaller than in the adult *A. opacum* and the pars nervosa is larger.

5. The postbranchial body resembles much that of other salamanders, but sometimes is shorter and lacking a lumen.

BREEDING EXPERIMENTS WITH CONFINED BREMUS (BOMBUS) QUEENS.[1]

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The biologist who attempts to give a complete account of the life-history and habits of the bumblebees of any part of the world, is generally confronted with the following difficulties: (1) He rarely, if ever, has the opportunity to study the beginning and early stages of a bumblebee colony, and (2) it is usually impossible for him to ascertain the biology of the less common species, because he is unable to secure their nests. The first attempts to overcome these difficulties were made by the Austrian zoölogist Hoffer ('82). This eminent bumblebee student confined a large number of *Bremus* queens in a museum at Graz, and thus was able to observe how the queen of *Bremus lapidarius* constructs her nest and the first egg-cell. However, none of these breeding experiments of Hoffer ('82, p. 413) produced a colony.

Better results along this line were obtained by the late F. W. L. Sladen ('12), who succeeded in rearing several colonies of *Bremus terestris*, a species which is very common in most parts of Europe. About the same time, similar experiments were carried out by the Danish biologist Lindhard ('12) with queens of *Bremus agrorum, distinguendus, hortorum, lapidarius, subterraneus, sylvarum*, and *terrestris*. With the exception of *Bremus hortorum* and *subterraneus*, at least one queen of each of these species started a nest, some of the resulting colonies later becoming self-supporting. In this country, Mr. Theodore H. Frison ('18) was equally successful in artificially rearing a colony of *Bremus auricomus*, a species concerning whose biology little was known up to that time.

I became interested in this subject during the summer of 1921 and decided to try similar artificial breeding experiments with our

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[1] Contribution from the Entomological Laboratory of the Bussey Institution, Harvard University, No. 222.

New England species of *Bremus*. During the following spring and summer, about fifty queens belonging to eleven [1] of the thirteen New England species listed by Franklin ('12/13, pp. 190, 191) were captured in the Arnold Arboretum, within the city limits of Boston. After being confined for various periods of time, at least one queen of each of these species became broody and oviposited, but self-supporting colonies were only produced by six.[2] The names of these six species, and the number of colonies obtained from each, are listed in the accompanying table:

TABLE I.

Species.	Number of Colonies.
1. *Bremus bimaculatus* Cresson	2
2. *Bremus impatiens* Cresson	1
3. *Bremus perplexus* Cresson	1
4. *Bremus separatus* Cresson	2
5. *Bremus ternarius* Say	1
6. *Bremus vagans* Smith	2

Before discussing the methods which were used in these breeding experiments, it seems desirable to describe briefly those employed by the earlier workers. Hoffer ('82) supplied his queens with nesting material and plenty of fresh flowers. Each queen was probably confined in a separate box. At first, Sladen ('12) also confined each queen separately, giving her an artificial nest and a liberal supply of honey and pollen, but he was unable to get a colony started in this way. He then placed two queens (of the same species) in each box, and this method yielded better results. However, Sladen (p. 131) found that one of the queens always killed her companion about the time the eggs were laid, and that the victorious queen invariably deserted the nest, unless she was supplied with one or more workers. In order to avoid this killing of queens, Sladen (p. 132) modified the experiment by confining one queen with one or more workers in each box. This also proved successful, even when the workers were of a different species.

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[1] *Bremus affinis, bimaculatus, borealis, fervidus, impatiens, pennsylvanicus, perplexus, separatus, ternarius, terricola,* and *vagans*.

[2] On June 13, several of these incipient bumblebee colonies were exhibited at a meeting of the Cambridge Entomological Club.

The methods employed by Lindhard ('12), differ in several respects from those of Hoffer ('82) and Sladen ('12). Lindhard (pp. 337, 338) used nest-boxes which were constructed as follows: Each nest-box consisted of two compartments of about 20x20x20 cm. each, one of which may be called the front compartment, or No. 1, and the other the rear compartment, or No. 2. On one side, compartment No. 1 had a glass pane to admit light. At the base, this compartment was provided with two flight-holes, *a* and *b*; *a*, communicating with the outside world, and *b*, with compartment No. 2. Both flight-holes could be opened and closed.

After lining compartment No. 2 with a layer of sod, about 5 cm. thick, Lindhard (p. 337) filled the interior with dry grass and the like. In some cases (cf. p. 341), a small paste-board box, filled with moss, and provided with a glass cover, was placed in the compartment instead of sod, and was surrounded with loose earth. With this arrangement, it was possible to place food in compartment No. 1 without disturbing the queen while she was engaged with her nest in the other compartment, and to keep the nest at a more uniform temperature.

As food, Lindhard (p. 337) provided a 50 per cent sugar solution and flowers, preferably those from which bumblebees were then obtaining pollen out in the open. After the queen had begun nest-building, the flight-hole was opened so that she could gather pollen from large bouquets which were put in the room in which the box was kept. As soon as the first worker emerged, the colony was placed out of doors where further development proceeded under normal conditions. In subsequent experiments, the queens were permitted to forage in the open shortly after they had begun nest-building. This method promised equally satisfactory results at the time Lindhard ('12) reported his work.

In his breeding experiments with *Bremus auricomus*, Frison ('18) confined two queens in one box, but, unlike Sladen (p. 131), found that they did not kill each other. As nesting material, Frison ('18, p. 44) supplied an old field-mouse nest in which he placed a honey-moistened lump of pollen. In addition to this, the queens were given a mixture of honey, rye-flour, and water.

The methods which were used in my own experiments, may be described briefly as follows: Each nest-box was provided with a double cover, the lower being made of glass and the upper of wood or tar-paper. At one end of the box, a round hole, ½ inch in diameter, which could be closed by means of a cork, served as a flight-hole. A small piece of honeybee foundation (wax), about an inch square, was then firmly pressed to the bottom of the nest-box near the opposite end. Around this piece of wax, a circular layer of cotton was placed as nesting-material. A tin can, about 3 inches in diameter and 2 inches high, from which the cover was first removed, was then put upside down over the honeybee foundation and cotton, after a hole, through which the queen could readily pass, had been made in the rim. Every two or three days, a fresh supply of pollen, obtained from two colonies of honeybees which had been especially secured for this purpose, was provided on the layer of wax within the cotton ring. Liquid food, consisting of about half water and half honey, was supplied daily in a porcelain dish, about ½ inch high, outside of the tin can. In order to keep the nest-box as sanitary as possible, a small pile of dry sand was put in one of the corners of the box.

Not being acquainted with the methods employed by Lindhard ('12), at the time my experiments were carried out, I at first placed two queens (of the same species) in each nest-box, as was done by Sladen ('12) and Frison ('18). However, like Sladen (p. 131), I found that one of the queens invariably killed the other,[1] sometimes within a few minutes after they had been placed together, and that the victorious queen was frequently made useless for further breeding experiments by the loss of one or more antennæ, or legs.[2] I therefore only placed one queen in each nest-box in subsequent experiments, and furnished each one with from one to three workers, preferably of her own species. Whenever a worker died—a rather frequent occurrence, especially as long as there was no brood—another was substituted as soon as possible. Those bumblebee "nuclei" which belonged to species easily obtainable in or near Boston, were permitted to forage out of doors, whenever the weather was pleasant, provided eggs or larvæ were present in the nest. Shortly after the first worker emerged, the tin can was removed, and the young colony, after being provided with additional nesting material, was given complete liberty.

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[1] As already stated, Mr. T. H. Frison ('18) found that two queens of *Bremus auricomus* behaved differently in this respect, but, judging from Mr. Frison's (p. 45) account, it seems probable that one of the queens was in poor health.

[2] These observations were made on about twenty queens of *Bremus bimaculatus, fervidus, impatiens*, and *vagans*.

Several nuclei, instead of building the first cells on the honeybee foundation, started their nests on the floor of the box, outside of the tin can. The behavior of such nuclei was as follows: The queen and workers, with outstretched abdomens, nestled closely to the floor of the nest-box about a certain spot which they had cleared of all foreign matter. As a result of this behavior, the chosen spot was gradually (sometimes within a day or two) coated with a layer of wax, and at this place the first cell was built. On several occasions, I tried to discourage the bees from starting their nest outside the tin can, by placing sand on the spot which they had selected. However, this did not disconcert them, for they immediately began to push the sand aside, picking up the larger grains with their mandibles and carrying them to the periphery of the wax-covered area. This experiment was repeated several times with a nucleus of *Bremus bimaculatus*, but the bees could not be persuaded to start their nest under the tin can until a layer of soap was substituted for the wax which they had deposited.

Having given a general account of the methods used in rearing bumblebee colonies from confined queens, I shall now proceed to discuss my own experiments somewhat more in detail. In order to make these data as complete as possible, a brief account of what is known concerning the nesting habits of our New England species has been added, with the exception of those cases where this has already been done in a previous paper ('22*b*). Since all of these breeding experiments were carried out during the spring and summer of 1922, the year has been omitted from most dates, 1922 being understood, unless otherwise indicated.

Terrestris GROUP.

1. *Bremus affinis* Cresson.

From the latter part of May until the end of June, several queens of this species were confined in separate nest-boxes with two or three workers. Although two of the queens constructed egg-cells and oviposited, the young larvæ died, apparently because they were not fed.

That it is possible to rear some, if not all, of our American *Bremus* species by the methods which were finally adopted by Lindhard ('12), is shown by the following incident, and another which will be discussed in connection with *Bremus vagans*. On May 26, a queen and two workers of *Bremus affinis* were confined together. Two days later, the queen constructed an egg-cell and oviposited, but on May 30, it became apparent that she had forsaken her brood. She was therefore set free from a third story window of one of the Bussey buildings, about noon on the following day, and her eggs and the two workers were turned over to another *affinis* queen. About five hours later, an *affinis* queen was noticed examining carefully several second story windows of the building referred to above, whereupon she mounted to the third story window from which the *affinis* queen had been liberated, and attempted to get in. I hastened upstairs, opened the window, and tried to catch her with my insect net, but missed her, and she flew away. About 11 A.M. on the following day (June 2), she again appeared at the third story window, but left as soon as I opened it, and, to my knowledge, did not return.

The nesting habits and disposition of *Bremus affinis* have been discussed in a recent paper ('22*b*), but the following incident seems worth recording. On June 2, a nest-box containing a queen, three workers, and an egg-cell of this species was accidentally jarred. Both, the workers and the queen immediately began to buzz angrily and rush out from beneath the tin can. In doing so, the queen accidentally encountered one of the workers, seized the latter and stung it to death.

II. *Bremus terricola* Kirby.

A queen of this species was confined on May 26, and a few days later three *terricola* workers were associated with her. Shortly after the introduction of the workers, the queen oviposited, and on June 16, the nest contained several small larvæ. About two weeks later (July 4), two exceedingly small *terricola* workers were noticed on the sand pile. They were unable to crawl, and had probably been released from their cocoons by the queen or workers and then dragged to the sand pile. No other brood being present in the nest, this nucleus was combined with another *terricola* colony which had been taken on the preceding day.

The nesting habits of this species have been dealt with in another paper ('22*b*).

Borealis GROUP.

I. *Bremus borealis* Kirby.

Four queens of this species, captured in or near the Arnold Arboretum, were experimented with. As no *borealis* workers could be obtained in the vicinity of Boston, two workers of *Bremus fervidus* were given to queen No. 1 (confined May 29), and three workers of *Bremus impatiens* to queen No. 2 (confined June 6), but neither one of the queens would coöperate with the foreign workers. On June 25, queen No. 1 was found dead in the nest, whereupon the workers of both queens were liberated, neither queen having started a nest. A somewhat different method was then resorted to. On June 26, about a dozen cocoons of *Bremus impatiens* from which workers were just beginning to emerge, were given to *borealis* queen No. 2. She immediately adopted both, the cocoons and the workers, and on June 28 constructed an egg-cell and oviposited. Two days later, this mixed colony was permitted to forage out in the open, after a small notch had been made in one of the wings of the queen. When the nest was examined on the following day, the queen was missing and did not return.

Borealis queen No. 3, captured July 2, was confined with sixteen worker cocoons of *Bremus impatiens*, which she adopted at once. On the following day, she built an egg-cell and oviposited. By July 5, four workers of *Bremus impatiens* had emerged, and this *borealis-impatiens* colony was also given complete liberty; but eight days later, the queen was found dead in a corner of the nest-box, probably as a result of an encounter with the workers.

Queen No. 4 was confined on July 8 with fourteen worker cocoons of *Bremus fervidus*, which were adopted immediately. She laid a batch of eggs on the following day, and another on

July 11. Meanwhile several *fervidus* workers had hatched, and, beginning July 13, the colony was allowed to forage in the open. For a time, this *borealis-fervidus* colony seemed to get along very well, but on July 19, it was noticed that the *fervidus* workers kept the *borealis* queen from the comb most of the time by daubing her with honey, a habit which has been described in another paper ('22*a*). In spite of this treatment, the queen lingered about the nest until August 18, when she died. During the first part of August, several *fervidus* males hatched in this nest, but no adult *borealis* were obtained from any of these mixed colonies.

As has been pointed out in another paper ('22*b*), practically nothing is known concerning the nesting habits of this species.

Pratorum GROUP.

I. *Bremus bimaculatus* Cresson.

After losing several *bimaculatus* queens through dueling, a queen of this species was confined alone on May 20. Two days later, a *bimaculatus* worker was given to her, but she squirted the latter with faeces,[1] and showed her hostility in other ways. On the following day, another *bimaculatus* worker was substituted for the first. With this second worker she soon became friendly, and by the next morning a honey-pot and a cell containing eggs were present in the nest. On May 26, two more *bimaculatus* workers were added to this nucleus. The first batch of larvæ—twelve in number—grew rapidly and began spinning their cocoons about June 7, and the first adult—a male—emerged on June 18. The bees which hatched from the remaining eleven cocoons, as well as those which emerged later, were likewise males. It is evident, therefore, that this *bimaculatus* queen had not been fertilized the preceding fall, and that, in some instances, bumblebee males may be produced as early in spring as workers, a fact which has been overlooked by other bumblebee students (cf. Dahlbom ('32, pp. 9, 10), Schmiedeknecht ('78, pp. 317, 320, 323), Hoffer ('82/83, p. 15), Wagner '07, p. 126), Sladen ('12, p. 49), and Stellwaag ('15, pp. 466, 467)).

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[1] This method of warfare is also employed by the queens and workers of other American species, *e.g.*, *Bremus impatiens*. In Europe, Wagner ('07, p. 82) observed a similar behavior in the case of *Bremus variabilis*, a queen of this species squirting the liquid for a distance of more than 35 cm.

Another *bimaculatus* queen and two workers were confined together on May 26. The first eggs were laid on June 5, and the first worker emerged on June 29, whereupon the colony was given complete liberty. On July 17, this colony consisted of the queen and 23 workers, a number of queens and males being produced later. The colony had completely died out by August 15.

The nesting habits and disposition of this species have been dealt with in two other papers ('22, '22*b*).

II. *Bremus impatiens* Cresson.

After several queens of *Bremus impatiens* had killed each other, a queen of this species was confined alone on May 20. By May 27, she had constructed an egg-cell and oviposited, and on the following day she proceeded to build a honey-pot, all of this work being done without the assistance of workers. On May 31, June 2, and June 3, respectively, three workers of *Bremus impatiens*—the first obtainable—were given to her, and by June 4, another honey-pot and two additional batches of eggs were present in the nest. On June 9, the first batch of larvæ—eight in number—were almost full-grown, and ten days later the first worker emerged, whereupon the flight-hole was left open permanently. When this colony was examined on August 15, it consisted of the queen, 122 workers, and a considerable quantity of brood. The colony broke up toward the end of September, after having produced a large number of males and young queens.

The nesting habits and temper of this species have been discussed in several other papers ('22, '22*a*, '22*b*).

III. *Bremus perplexus* Cresson.

A queen and two workers of this species were confined on June 6, and another queen and three workers on June 11. Both nuclei began nest-building on the day on which they were confined, but on June 17, queen No. 2 and one of her three workers were found dead in the nest, whereupon the remaining two workers and brood were given to queen No. 1. The larvæ grew rapidly, and on June 29,—twenty-three days after queen No. 1 was confined—the first worker emerged.[1] Several others hatched during the next few days, and on July 1, the colony was given its liberty. In order to give it a better start, about twenty cocoons of *Bremus impatiens* were placed in the nest. The workers of the two species showed no hostility toward each other, and everything went well until July 14, when the *perplexus* queen was found dead in the nest. Like *borealis* queen No. 3, she probably was killed by the *impatiens* workers. During the first half of August, this *perplexus-impatiens* colony produced several males of both species, but by August 20, the colony had completely broken up.

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[1] This confirms the observations of Sladen ('12, p. 31) and Frison ('18, p. 47), who found that it takes from 22 to 25 days for the workers to emerge, from the time the eggs are laid. Hoffer's (82–83, p. 28) claim that the development of the workers, from egg to adult, takes a month, is therefore incorrect.

What is known about the nesting habits of *Bremus perplexus*, we owe to Franklin ('12/13, pp. 347–348). Some years ago, this author took two nests in early August, in Vermont. Both nests were situated in the walls of houses, and were made of wool. One of the nests contained 5 queens, 1 male, and 9 workers; and the other, 8 queens and 33 workers.

In the vicinity of Boston, *Bremus perplexus* is very rare. Judging from the early appearance of the workers (the first one was taken on May 28), some queens of this species must appear as early as May 1, and most nests are probably started during that month. The sexual forms seem to be produced chiefly during July and August. The nests probably break up in September.

Regarding the disposition of *Bremus perplexus*, Franklin (p. 348) has the following to say: "This is the gentlest and least ready to sting of all the bumblebee species which I have had to deal with in the living condition. This seems peculiar, as *B. vagans*, which seems to be its nearest ally, is exceedingly ferocious." I have already ('*22b*) taken exception to the last part of this statement. According to my observations, *Bremus perplexus* and *Bremus vagans* are similar in disposition, both species being comparatively gentle.

IV. *Bremus ternarius* Say.

Of this species, two queens were taken on *Rhododendron*, in the Arnold Arboretum, on June 6, and June 8, respectively. Besides having lost much of her pile on the dorsal side, *ternarius* queen No. 2 had a very distended abdomen, suggesting that she probably had already started a colony. She was therefore set free a few minutes after she was captured with the hope that she might furnish workers for *ternarius* queen No. 1. As queen No. 1

showed no interest in the nesting material, three workers of *Bremus bimaculatus* were placed in her nest-box on June 14, but she would have nothing to do with them, and three days later, two small workers of *Bremus impatiens* were substituted for the three *bimaculatus* workers. With these two workers, the *ternarius* queen made friends and a few days later began nest-building. The first batch of eggs was laid about June 21, and the first *ternarius* worker hatched on July 14. By July 16, six more workers had emerged. The two workers of *Bremus impatiens* were then removed, and the young *ternarius* colony was left to shift for itself. By August 13, the number of workers had increased to seventeen, and a few weeks later, several newly-hatched *ternarius* males were present in the nest. At the beginning of September, the queen showed signs of becoming feeble, and on September 10, disappeared from the nest. The last workers died during the first week of October.

Very little is known concerning the nesting habits of *Bremus ternarius*. During the summer of 1863, Putnam ('64) took a nest [1] of this species at Bridport, Vt., on the borders of Lake Champlain. It was situated either under an old stump or under the clapboards of a house.

In the vicinity of Boston, *Bremus ternarius* is exceedingly rare. The queens, like those of *Bremus vagans*, seem to leave their winter quarters comparatively late in the spring. Most nests are probably started between the 15th of May and the 15th of June. If this assumption is correct, the first workers ought to appear shortly after June 1. As in most other New England species, the males and queens are probably produced chiefly during August and September.

Putnam (p. 99) states that *Bremus ternarius* is far more savage than *Bremus fervidus*, the latter species, according to this author, being "of quite a gentle disposition." However, I found both of these species to be extremely vicious. In other respects, the behavior of *Bremus ternarius* reminds one very much of that of *Bremus perplexus* and *Bremus vagans*.

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[1] The other nest which Packard ('64) and Putnam ('64) considered as belonging to *Bremus ternarius*, according to Franklin ('12/13, pp. 444-445), was probably a nest of *Bremus rufocinctus*.

V. *Bremus vagans* Smith.

As in the case of *Bremus bimaculatus* and *Bremus impatiens*, several queens of this species were at first lost through dueling. The two queens from which self-supporting colonies were obtained, were confined—each with three workers—on June 8, and 11, respectively. Within a week, both nuclei had begun nest-building, and by the end of June the first batches of larvæ were spinning their cocoons. The first worker of nucleus No. 1 hatched on July 13, and the first one of nucleus No. 2, on July 14, whereupon both nuclei were given continuous liberty. The two colonies prospered and did not break up until the latter part of September, each having produced a number of queens and males.

As already mentioned in connection with *Bremus affinis*, a confined bumblebee queen, if liberated, may return to an artificial nest after she has oviposited in it. From the following incident, it will be seen that *Bremus vagans* is no exception to this rule. On June 22, the weather was exceptionally pleasant, and *vagans* nucleus No. 2 having small larvæ, the flight-hole of the nest-box was opened at about 9 A.M., in order to give the bees a chance to forage. When the nest was examined at noon, the queen, as well as the workers, had disappeared. At 2 P.M., none of the bees had returned, and, believing they had forsaken the brood, the nest-box was removed with the intention of turning the young larvæ over to *vagans* nucleus No. 2. However, about 5 P.M., *vagans* queen No. 1 was found eagerly searching about the place where the nest-box had been. She was captured, and upon being placed in the nest-box, quickly went to her brood. The workers did not return, and three others were substituted on the following day.

The nesting habits and disposition of *Bremus vagans* have been discussed in two recent papers ('22, '22*b*).

Auricomus Group.

I. *Bremus auricomus* Robertson.

This is one of the two species which I was unable to obtain in the vicinity of Boston. All that is known concerning the nesting habits of *Bremus auricomus*, we owe to the efforts of Mr. Theodore

H. Frison ('17, '18, '21). In addition to the colony which he reared artificially, Mr. Frison ('17, '21) had under observation several nests of natural origin, one of which was taken on September 6, 1917. It was situated in a hollow cement block in the foundation of a small cabin, and contained 3 young queens, 3 males, and 15 workers—10 living and 5 dead—besides several others which were out foraging when the nest was taken. Another nest, examined July 26, 1919, at Clyman Junction, Wis., was situated about 1½ ft. below the surface of the ground, and contained the old queen, 12 workers, 15 eggs, and some larvæ and pupæ of *Bremus auricomus* as well as a disabled *Psithyrus laboriosus* queen. In addition to these three colonies, Mr. Frison ('17) had under observation another which was started in an artificial nest which had been placed in a clay embankment.

According to Mr. Frison ('18), *Bremus auricomus* is rather gentle in disposition. Concerning the colony which he took on September 6, 1917, he has the following to say: "The bumblebees were very docile when the nest was removed, for instead of flying angrily from the nest, the most they did was to run excitedly about on the comb and buzz loudly."

Fraternus GROUP.

I. *Bremus rufocinctus* Cresson.

As in the preceding case, I was unable to obtain queens of this species in the vicinity of Boston. Comparatively little is known about the nesting habits of *Bremus rufocinctus*. According to Franklin ('12/13, pp. 444–445), Putnam ('64) took a nest of this species at Bridport, Vt., in September, 1863. It was probably situated under the clapboards of a house, about eight feet from the gound, and contained 28 adult bees and 35 cells with young.

Judging from Putnam's (p. 99) account, *Bremus rufocinctus* is one of the more savage species.

II. *Bremus separatus* Cresson.

On May 15, a queen of this species which had been captured at Peabody, Mass., was turned over to me by Dr. L. H. Taylor. She had lost a part of one of her antennæ and, although given a *separatus* worker, refused to take any interest in the nesting material. She was found dead in the nest-box on June 9.

Queen No. 2 was taken on June 3. Having lost both of her antennæ, she took little interest in life and died five days later.

Queens No. 3 and No. 4 were taken in the Arnold Arboretum on June 8, and June 16, respectively. They were confined separately, and each one was given three workers. Both of these nuclei at once started nest-building, and toward the end of June each had large larvæ. The first workers emerged on July 9, and 11, respectively, whereupon both colonies were given their liberty. A few days later, queen No. 4, returning from a foraging trip, by mistake entered a nest of *Bremus affinis* and was stung to death. Her brood and workers were given to *separatus* colony No. 3. This colony prospered and produced a number of males and queens in August, but had completely died out by September 10.

According to Putnam ('64), *Bremus separatus* builds its nests "under old stumps and in other situations similar to those in which the nests of *B. fervidus* are found."

In regard to the disposition of *Bremus separatus*, Putnam (p. 101) has the following to say: "This species is nearly as ferocious, on being disturbed, as *B. ternarius*," a statement which is corroborated by my own experience.

Dumoucheli GROUP.

I. *Bremus fervidus* Fabricius.

After losing several queens of *Bremus fervidus* by dueling, a queen of this species was confined alone on May 24, but she refused to start a nest. On June 2, three *fervidus* workers were associated with her, and three days later the nest contained two honey-pots and a closed egg-cell, but the larvæ which hatched from the eggs died, apparently because they were not fed by the adults. Several other *fervidus* nuclei which were started later, likewise paid no attention to their larvæ.

The nesting habits and disposition of *Bremus fervidus* have been discussed in several recent papers ('22, '22*a*, '22*b*).

II. *Bremus pennsylvanicus* De Geer.

A queen of this species was confined on May 29, and six days later, three workers of *Bremus fervidus* were given to her, but she remained restless and would have nothing to do with them.

Another *pennsylvanicus* queen was therefore put in her place on June 5. Although hostile to the *fervidus* workers, queen No. 2 constructed an egg-cell and oviposited on June 11. But, as in the case of the *fervidus* nuclei, the larvæ were not fed and died shortly after hatching. Both *Bremus fervidus* and *Bremus pennsylvanicus* are Pocket-makers, *i.e.*, they feed their larvæ, at least those of the workers,[1] through one or more pockets which they make at the side of each group of larvæ. On returning from the field, the foraging bee deposits its load of pollen directly into these pockets, through which the latter reaches the larvæ. It seems probable, therefore, that the Pocket-makers let their worker larvæ die, whenever they cannot feed them in the usual way. If this supposition is correct, it will be impossible to rear colonies of the Pocket-makers from confined queens, unless the latter are permitted to collect pollen from flowers.

Since the methods employed in rearing colonies of other species yielded no results in the case of *Bremus fervidus* and *Bremus pennsylvanicus*, and as I was anxious to obtain a colony of the latter species, a different method was resorted to. On June 26, about a dozen cocoons of *Bremus impatiens* were given to *pennsylvanicus* queen No. 2, which she adopted immediately. She showed no hostility toward the young workers which emerged, and two days later constructed an egg-cell and laid a batch of eggs. On July 2, this mixed colony was placed out of doors so that the workers could forage. Everything went well until July 6, when the queen was found dead in the nest, having probably been killed by the *impatiens* workers.

On July 26, a third *pennsylvanicus* queen was confined with a *pennsylvanicus* worker and sixteen cocoons of *Bremus fervidus*, and on August 2, another *pennsylvanicus* worker was added to this nucleus. The cocoons were adopted immediately, as were the *fervidus* workers which hatched from them. On August 3, the queen built an egg-cell and oviposited, and two days later this *fervidus-pennsylvanicus* colony was given its liberty. *Pennsylvanicus* worker No. 1 did not return, but No. 2 and several of the *fervidus* workers brought in one load of food after another.

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[1] The queen and male larvæ of *Bremus fervidus*, and probably also those of *Bremus pennsylvanicus*, are fed, at least toward the end of their development, like the larvæ of those bumblebees which do not feed their larvæ through pockets.

For several days, everything went well, but on August 10, it was noticed that the *fervidus* workers were daubing the *pennsylvanicus* queen and worker with honey, a habit which has been referred to before. On the next day, the *pennsylvanicus* worker failed to return, but the queen, although her pile was constantly soaked with honey, lingered about the nest until August 21, when she disappeared. A few days later, several *fervidus* males hatched in this colony, but no adults of *Bremus pennsylvanicus* were obtained from any of these mixed colonies.

The nesting habits of *Bremus pennsylvanicus* have been described by Franklin ('12/13) Howard ('18), and Frison ('16, '17, '18, '21). Judging from the data published by these authors, the nests are usually situated on the surface of the ground, but occasionally also in the ground, or in birds' nests. The largest nest taken by Franklin (p. 405) contained 1 queen, 23 males, 53 workers, and 78 cells with larvæ in them, of which 18 were queen cells.

In the vicinity of Boston, *Bremus pennsylvanicus* is comparatively rare. The queens are the last to appear in spring, the first one in 1922 being seen on May 29, and the first worker on July 22. Most nests are probably started in June. A number of males of this species were taken in September, and therefore the colonies, like those of *Bremus fervidus*, probably do not break up until the latter part of September or the beginning of October.

According to Mr. T. H. Frison ('17, '18) and Mr. Court W. Ranslow (cf. Howard, '18), the workers of *Bremus pennsylvanicus* are rather vicious. After they had oviposited, this was also true of the *pennsylvanicus* queens used in my breeding experiments. On several occasions, they seized my forceps, tried to sting them, and clung to them so tenaciously that they could be lifted out of the nest-box.

While these experiments were in progress, a number of other observations were made which will be presented in another paper.

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